Role of VEGFA, CXCR4 and VHL mutation in tumour behaviour

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Chapter 2

Role of chemokines and their receptors in cancer

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Current Pharmaceutical Design 2009;15:3396-416.

ABSTRACT

Metastases are the cause of 90% of human cancer deaths. The current treatment of cancer with chemo,- and/or radiotherapy is based on cell death by DNA damage neglecting the fact that cancer cell invasion into surrounding tissues and metastasizing are fundamental features of neoplasms and the major reason for treatment failure.

Metastasis is the result of several sequential steps and represents a highly organized, non-random, and organ-selective process. A number of *in vitro* and *in vivo* models show that tumour cells use chemokine-mediated mechanisms during this metastasizing process, comparable to those observed in the regulation of leukocyte trafficking. Furthermore, chemokines modulate tumour behaviour such as the regulation of tumour-associated angiogenesis, activation of host tumour-specific immunological responses, and direct stimulation of tumour cell proliferation in an autocrine fashion.

These findings may lead to new drugs that target chemokines or their receptors and will likely be of great additional value for treatment of cancer patients.

1. INTRODUCTION

Chemokines are small chemotactic cytokines which are characterized by their ability to induce directional migration of cells towards a gradient of chemokines (chemotaxis) by binding to a chemokine receptor. Invading pathogens such as *Staphylococcus aureus bacteria* can induce the secretion of chemo attractants resulting in a tissue gradient and direct leukocyte migration to the site of infection and inflammation. Interestingly recently striking similarities between this leukocyte trafficking and tumour cell migration revealed that they are both critically regulated by chemokines and their receptors (1). Also during organogenesis, directional migration is guided by chemokines and is important for the proper positioning of traveling progenitor cells that express chemokine receptors. Tumour cells turn out to express also functional chemokine receptors to sustain proliferation, angiogenesis, and survival and to promote organ-specific localization of distant metastases (2-4). This is of interest as current treatment of cancer with chemo,- and or radiotherapy is largely based on cell death by DNA damage neglecting the fact that invasion into surrounding tissue and metastasizing are fundamental features of neoplasms and the major reason for treatment failure.

The aim of this review is to provide an overview of the structure, activation and downstream signaling pathways and the function during development, infection and immunity of different chemokines and their receptors. Furthermore, we describe the chemokine-mediated mechanisms employed to modulate tumour cell behaviour. Finally, first interesting attempts to exploit this new area of anticancer treatment for anticancer treatment are addressed.

2. CHEMOKINES AND THEIR RECEPTORS

2.1. GPCR Structure and Ligand

G protein-coupled receptors (GPCRs) comprise a large protein family of transmembrane receptors that sense extracellular molecules and activate inside signal transduction pathways leading to cellular responses. GPCRs in general are seven-transmembrane receptors with seven helical membrane-spanning regions connected by six extramembrane loops (5). The N-terminus and the adjacent three loops are the extracellular domain, while the C-terminus and three remaining loops extend intracellular. Specific GPCRs possess the ability to bind a variety of specific ligands such as chemokines, light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters. The ligand binding region of the GPCRs varies per type of stimulus. Lipidderived stimuli (e.g. LTB4 and PAF) and small peptides (e.g. fMLP) primarily activate GPCRs through the transmembrane regions (6-8). A two-step binding model has been proposed for larger protein stimuli like chemokines. Chemokines bind the N-terminus of the chemokine receptors, which is necessary for proper receptor activation (9, 10). The GPCRs sensitive to chemokines are called chemokine receptors.

2.2. GPCR Activation by Chemokines

Chemokines are a large family of small proteins of 8 to 12 kDa that attract a variety of effector cells (see Table **1**). Chemokines have a low sequence homology but all show conserved structural homology (see Table **2**). They are divided in four groups based on the number and relative position of their cysteine residues (see Fig. **1**) (9, 11). The two large families of chemokines are the CXC- and CCchemokines, whereas the CX3C- and the XC-chemokines represent the two smaller families of chemokines. The CXC chemokines are subdivided into two subfamilies, based on the presence or absence of a Glu-Leu-Arg (ELR) sequence preceeding the first cysteine residue. ELR+ chemokines preferentially attract neutrophils, whereas ELR- chemokines act on lymphocytes. The CC chemokines are involved in the attraction of various cell types, e.g. monocytes, eosinophils, and lymphocytes.

Cells expressing chemokine receptors have the ability to migrate towards a chemokine gradient (see Fig. **2**). Interaction with these receptors results in the transduction of a signal and thus the activation of effector cells. Chemokine binding by GPCRs varies from highly specific to highly promiscuous. Until now, 7 receptors are known for CXC chemokines (CXCR1-7), 11 receptors for CC chemokines (CCR1-11), and one for CX3CL (CX3CR1) and XCL1 (XCR1). Chemokines not only bind to CXCR, but they also interact with glycosaminoglycans (GAGs) through distinct sites. These GAGs are long unbranched polysaccharides consisting of a repeating disaccharide unit and allow presentation of chemokines by endothelial cells or extra cellular matrix (ECM). Thereby, they create a chemotactic gradient that directs leukocyte as well as tumour cell transmigration through the endothelial layer towards the underlying tissue (see Fig. **3**).

2.3. GPCR Signaling

Upon ligand binding, GPCRs are activated and the signal is transduced to enable effector functions (12) (see Fig. **4**). GPCRs are coupled to heterotrimeric G proteins that are associated with their intracellular loops. Ligand binding induces exchange of GDP for GTP by the Gα-subunit, resulting in Gβγ-subunit dissociation. The Gβγ-subunit in its turn activates the phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PLC) leading to the activation of the mitogen-activated protein kinases (MAPK) pathway and accumulation of inositol trisphosphate and diacylglycerol in the cytoplasm, respectively. These products induce mobilization of calcium from intracellular stores and protein kinase C (PKC) activation which drive downstream cell signaling. The Gα-subunit also transduces the signal by interacting with adenyl cyclase, which is responsible for the synthesis of cyclic-AMP from ATP. This results in changes in the cellular responses and gene expression. Generally, activation of the signaling pathways leads to actin polymerization, which is responsible for cell shape change and chemotaxis. Effector cells can degranulate and secrete oxygen radicals crucial for killing of pathogens. Importantly, chemokine signaling induces the expression and activation of integrins on the leukocyte cell surface, allowing for firm adhesion of the leukocytes during extravasation from the blood vessel to infected tissues. In parallel with their role in controlling cell migration during immune responses, chemokines and their receptors have been implicated in the cancer metastatic process by directing the migration of tumour cells.

3. CHEMOKINE FUNCTION

3.1. Development

3.1.1. Pre-Implantation Embryo

Recent years, accumulating data has shown that chemokines play important roles during the first stages of embryonic development. For an embryo to develop, the conceptus has to implant into the endometrium. The implantation stage can be subdivided into three distinct phases: apposition, adherence and invasion, and accumulation of leukocytes. During apposition, the blastocyst approximates the endometrium where the conceptus and maternal cells communicate by the release of soluble factors such as chemokines and growth factors. Subsequently, the conceptus adheres to the endometrium and invades through the epithelial basement membrane and the decidualized stroma in order to attain its blood supply from the maternal vasculature (13). The accumulation of leukocytes in the endometrium, predominantly macrophages and uterine natural killer (uNK) cells, are particularly important for creating a permissive environment for the embryo during the periimplantation phase (14). These leukocytes release multiple soluble factors including chemokines and create a unique immunological environment that provides protective immunity, maternal tolerance and regulates the invasion of fetal cells (15). In the endometrium, macrophages comprise approximately 20% of the leukocyte population and are accumulated in regions of decidualization and trophoblast invasion (16). uNK cell numbers increase in the mid-luteal postovulatory phase in the menstrual cycle and amass around maternal spiral arterioles where these play important roles in shaping the spiral arterioles and in decidualization (17, 18). The leukocyte accumulation in the endometrium is tightly regulated and likely driven by chemokines. Decidualized stroma cells produce chemokines, such as CCL7, CCL21, CCL22, CCL4 and CX3CL1, all strong attractants for uNK cells that express receptors for these ligands (19, 20). Furthermore, decidual chemokines are key factors for the positioning of leukocytes around maternal spiral arterioles (21-24). Various chemokines and chemokine receptors are also present in the trophoblast during endometrium invasion, suggesting a contribution for chemokines in this process (23, 25-27). Disturbances in cytokine production have been detected in women who are infertile or who experience recurrent miscarriages (28). These findings illustrate the general importance of chemokines and their broad range of actions ranging from embryonic development to carcinogenesis.

3.1.2. Hematopoiesis

In mammals, primitive hematopoietic stem cells (HSC) are present in the yolk sack. They travel into the fetal liver and at the end of the second trimester the HSC move from the liver towards the bone marrow tissue (29). Mice deficient in CXCL12 or CXCR4 have a defective B-cell lymphopoiesis and impaired bone-marrow myelopoiesis (30, 31). Interestingly, myeloid development in the fetal liver of these mice remains largely unaffected, suggesting that chemoattractants other than CXCL12 contribute to the migration of HPCs during this developmental period. At the end of the second trimester bone marrow release of CXCL12 is highly increased and attracts CXCR4 bearing HPCs. The CXCL12/CXCR4 system remains active during adult hematopoiesis. In the clinic the mobilization of HPCs, used for transplantation in haematological disease, is stimulated by G-CSF

which reduces CXCL12 levels and increases CXCR4 expression in the bone marrow (32). In addition, CXCL12 promotes myeloid progenitor cell (MPC) survival. These findings are corroborated by the fact that transgenic mice endogenously expressing SDF-1/CXCL12 in all tissues have an enhanced MPC proliferation in the marrow and spleen and live longer after growth factor withdrawal (33, 34). In contrast to CXCL12, the effects on migration and survival/proliferation of a number of CC and CXC chemokines, including CCL3, CXCL5 and CXCL8, display myelosuppressive effects (35).

3.1.3. Chemokine Expression During Brain Development

Embryonic development comprises the generation of progenitor cells that subsequently migrate to their target location and finally differentiate into organotypic tissues. Studies regarding brain and spinal cord development have mostly been focused on the functions of CXCR4 and its ligand CXCL12. In the mouse nervous system CXCR4 expression starts from embryonic day (E)8.5 and remains present until adulthood. Early during development CXCR4 is accumulated in neural epithelium in an anterior to posterior wave (36). In the spinal cord and hindbrain, CXCR4 expression is evident at E10.5 and is localized in cells innervating muscle and viscera, with highest CXCR4 transcript levels in the dorsal root ganglia and ventral mantle layers of the spinal cord. In midbrain and forebrain structures expression of CXCR4 is most prominent between the midbrain/hindbrain border. During the formation of the cerebellum CXCR4 transcripts are detected starting with a wide expression pattern at E11.5 and the expression is later confined to cells in the external granular layer (31). At around E14.5 both CXCL12 and CXCR4 appear in the developing hypothalamus, thalamus and in cells at the border surrounding the olfactory bulb (36). Later at E15.5, CXCL12 and CXCR4 transcripts are also present in the neo-cortex, the hippocampus and in the meninges and expression levels are sustained into the adult central nervous system (CNS). Notably, CXCR4 and its ligand CXCL12 show a complementary expression pattern in both the developmental prenatal and perinatal to adult period emphasizing their putative role in migration, proliferation and survival (31, 37-40).

3.1.4. Chemokines and Neuronal Migration

The meninges covering the developing neocortex release CXCL12, which acts as a chemoattractive cue for the CXCR4 expressing Cajal-Retzius (CR) cells and regulates their tangential migration in a paracrine manner (41-42). These CR cells, a transient population of neurons, which are located in the marginal zone adjacent to the meninges, largely regulate cortical layer formation by secreting the glycoprotein reelin. This protein maintains radial glia cells acting as a physical scaffold that allows newborn neurons to migrate the long distance from the ventricular zones towards the pial surface (43, 44). Cortical projection neurons or pyramidal cells migrate into the neocortex from two important proliferative zones termed the ventricular zone (VZ), containing dividing cells at the ventricular surface, and the sub-ventricular zone (SVZ) that contains neuronal progenitor cells (45). Pyramidal cell progenitors migrate according to an inside-out sequence: meaning that the most recently generated neurons migrate towards the pial surface through previously established layers of cells (46).

Accordingly, neurons in layer II are generated at a later time period than layer IV and V neurons. Because of the CXCR4 expression by CR cells and CXCL12 release in the neocortex, it was proposed

that CXCL12/CXCR4 contribute to the proper lamination pattern of the cortex early in cortical development. Although smaller in sheer numbers, the second neuronal population inhabiting the cortex are the GABAergic interneurons that give local input to pyramidal cells as well as other interneurons. In developing embryos immunolabeling for GABA in combination with fluorescence dye labeling of cultured telencephali identified streams of migrating GABAergic cells undergoing long distance tangential migration to reach their final position in specific cortical layers (47, 48). In the mouse, GABAergic interneurons originate in the subpallial telencephalon starting from around E12 and follow precise migration pathways towards the cortex (49). The first indication that CXCR4 is involved in migration of cortical interneurons was demonstrated by *in situ* hybridization studies in CXCR4 and CXCL12 knockout mice. In these mice the developing cortex showed a disturbed localization of neurons expressing the enzyme glutamic acid decarboxylase, a specific marker for GABA producing cells. In addition, CXCR4 is expressed in migrating GABAergic precursors which are attracted towards a gradient of CXCL12 (42, 50). CXCL12 is expressed by proliferating pyramidal cell precursors and not in an autocrine manner by tangentially migrating interneurons (51).

The timing of exit from the migratory streams is accompanied by a loss of responsiveness to CXCL12 as chemoattractant (52). In cortical areas with a reduced interneuron density the development of a functional cortical circuitry and the inhibitory tone regulated by GABAergic cells was altered. CXCL12 is also expressed by the meninges surrounding the dentate gyrus (DG) of the hippocampus during its early development (37, 38). A mixture of proliferative and post-mitotic dentate granule cells initially migrates from the dentate neuroepithelium towards the DG anlage and determine the morphology of the primary dentate gyrus and hilus. CXCR-/- mice have ectopically located DG cells near the ventricular zone, adjacent to the fimbria or in the migratory stream towards the dentate anlage. Furthermore, explant assays revealed that CXCL12 directly regulates DG cell migration (37). The DG of the hippocampus is an important site of adult neurogenesis and the expression of CXCR4 and CXCL12 is continued until adulthood. This raises the possibility of a continuing role in the maintenance of adult-born granule neurons. Similar to the neocortex and the DG, the development of the cerebellum relies on CXCR4/CXCL12 signaling pathways. Granule cell precur sors originate from the upper rhombic limb and migrate tangentially to form the external granular layer. Here, the granule cells proliferate extensively and subsequently migrate towards the internal granular layer (53). CXCR4 is expressed by granule cell progenitors that are attracted to CXCL12 released by the meningeal cells (40). In CXCR4-/- mice the gross anatomical development of the cerebellum is unaltered, however the laminar structure is aberrant. Clusters of granule cells are positioned ectopically underneath the Purkinje cell layer or are intermingled with Purkinje cells (31). Similar developmental deficits were observed in CXCL12-/- mice (39). The above processes and the role of CXCR4/CXCL12 in particular in these could have important implications for various neurodevelopmental disorders.

3.1.5. Chemokines and Axon Guidance

Neuronal circuitries are formed by neurons that sprout elongating axons and dendrites with at their tips a highly motile structure called the growth cone. Growth cones are densely loaded with cell surface receptors that interpret the presence of guidance cues through alterations in the actin

cytoskeleton and thereby induce an attractive or repellent response (54). By this means axons and dendrites are steered towards their appropriate locations (55). The effects of CXCR4/CXCL12 on neuronal migration and the common molecular mechanisms shared between migration and axon guidance raises the possibility of a role for chemokines in axon guidance (56). Xiang *et al.* were first to demonstrate that growth cones of cerebellar neurons could be steered by a gradient of chemokines, including CXCL12 (57). The establishment of an *in vitro* gradient of CXCL12 induced an attractive or repellent response dependent on the differential activity of the PKC and IP3 pathways. Their findings were later corroborated in cultured neurons derived from the hippocampus and the cerebellar granule layer (58, 59).

Moreover, motor neurons that extend axons out of the neural tube to innervate their target muscle express axonal CXCR4 and are attracted towards CXCL12 released by mesenchymal cells surrounding the ventral neural tube (60). The effects of CXCL12/CXCR4 signaling are dependent on Rho-GTPase activation. At low concentrations CXCL12 promotes axon elongation, however at higher concentrations Rho/ROCK activity repressed axonal growth (58). CXCR4 also elicits an increase in intracellular Ca2+ and activation of MAPK, two pathways known to influence axon guidance (61, 62).

3.1.6. Other Chemokines in Neuronal Development

Increasing evidence suggests that other chemokines also play an important role in nervous system development. CXCR2 and its ligand CXCL6 are expressed in the developing rat midbrain (63). CXCR2 mRNA is expressed at the onset of dopaminergic neurogenesis and peaks at E13.5 with the birth of DA neurons. The CXCR2 ligand CXCL6 was first detected at E11.5 and was present until postnatal day (P)1. *In vitro* CXCL1, CXCL6 and CXCL8 regulate via CXCR2 the proliferation of dopaminergic progenitors, neurogenesis and the differentiation of precursor cells into ventral midbrain dopaminergic neurons. Besides neurons, the nervous system depends on supporting cell types such as oligodendrocytes which myelinate axons in each region of the developing CNS. In the spinal cord, oligodendrocyte precursors cells (OPCs) migrate the long distance from the ventricular zone to white matter where they reach their final destination. Although most chemokines act as chemoattractants in the developing CNS, the migration of immature OPCs was found to be inhibited by the CXCL1/CXCR2 axis (64). CXCL1 reduces the number of cells in the dorsal spinal cord and in CXCR2-/- mice have less differentiated oligodendrocytes with an altered distribution.

3.1.7. Chemokines in other Organs Systems

Chemokines are also involved in cardiovascular development. Starting as a common outflow tract, the formation of the septum establishes separate aortic and pulmonic outflow racts. The aortopulmonary septum further fuses with inferior muscular portion of the ventricular septum. *In situ* analysis has shown that the aortopulmonary cardiac septum expresses CXCR4, while the ventricular muscle and outflow tract express CXCL12 (36). This matches findings in CXCL12-/- and CXCR4-/- embryos, which display a cardiac ventricular septum defect (30, 31). In addition to cardiac development, CXCR4 is critical for the vascularization of the gastrointestinal tract. Areas of vascularization correlate with high CXCL12 expression and CXCR4 transcripts are present in the endothelium of developing blood vessels during embryogenesis (36). In CXCR4-/- mice the

major blood vessels such as the aorta, vena cava, carotid artery and jugular vein are present, but mesenteric vascular branching is aberrant leading to hemorrhages or congestion of the small intestines (65). It is clear that chemokines contribute to the pathogenesis of renal disease and in particular renal inflammation (66). However, data regarding the functions of chemokines during kidney development is still scarce. CXCR3, CX3CR1 and its ligand fractalkine, and CXCR4 and its ligand CXCL12 are present in different renal compartments with diverging temporal expression during kidney development (67). These tightly regulated spatially and temporally expression patterns and the knowledge that these chemokine affect adhesion, proliferation and have vascular effects suggest their involvement in embryonic renal development.

3.2. Infection and Immunity

For successful infection of the host, invading pathogens first need to cross the mucosal surfaces and the skin. Upon subsequent entrance of host tissue by the pathogen, the acute inflammatory response of the innate immune system is initiated. Neutrophils and macrophages are the main initial effector cells of the innate immune system that can clear the invading pathogen by phagocytosis. These cells are attracted to the site of infection through sensing of chemotactic factor gradients. Chemoattractants are secreted by activated host cells and released as activated complement components upon recognition of evolutionary conserved structures presented by pathogens. The secreted chemoattractants form a gradient in the tissue, thereby directing phagocyte migration to the site of infection and inflammation. Well-known chemoattractants for phagocytes are host-derived platelet activating factor (PAF), leukotriene B4 (LTB4), complement fragment C5a, and chemokines such as CXCL8 (also known as interleukin (IL)-8). Bacterial-derived products also serve as effective chemoattractants. These include formyl peptides such as N-formyl-methionyl-leucyl-phenylalanine (fMLP). All these chemoattractants activate phagocytes by binding to GPCRs. Activation through GPCRs not only directs phagocyte chemotaxis but also primes and activates the cells for effector functions such as phagocytosis.

Chemoattractants and GPCRs thus play an essential role in directing the innate immune defense against invading pathogens. It is therefore not surprising that pathogens have evolved a large variety of strategies to evade activation of phagocytes by chemoattractants. Several of these bacterial proteins were identified; CHIPS (68), staphylococcal complement inhibitor (SCIN) and its homologues (69, 70), FPRL1 inhibitory protein (FLIPr) (71), staphylococcal superantigenlike 5 (SSL5) (72, 73) and staphylococcal superantigen-like 10 (SSL10) (74).

3.3. Cancer and the Chemokine Network

3.3.1. CXCR4- CXCL12

CXCR4 is the most widely expressed chemokine receptor in many different haematological and solid cancers (75) and has been associated with metastases and poor prognosis (76-80). Metastasizing is the result of several sequential steps and represents a highly organized, non-random and organselective process. Increasing evidence suggests a pivotal role of CXCL12

and its receptor CXCR4 in this process (1). CXCR4 is highly expressed in human primary breast cancer cells and metastases. CXCL12 exhibits peak expression levels in organs representing the first destination of breast cancer metastasis. *In vivo*, neutralizing the interactions of CXCL12/CXCR4 impairs metastasis of chemotactic and invasive responses (81). CXCR4 is also involved in metastasizing of prostate cancer cells to the bone marrow (4) and colon cancer cells to the liver (82). In glioblastomas (GBM) and neuroblastomas CXCR4 is expressed and CXCL12 is produced, creating autocrine loops. In these tumours the expression of CXCR4-CXCL12 correlates positively with tumour cell proliferation (83-86) and in neuroblastomas particularly there is a positive correlation between increased expression of CXCR4-CXCL12 and tumour progression and metastasis (84). In GBM cells, increased CXCR4 expression was related with increasing tumour grades (86, 87). In addition, CXCL12 provides survival signals to CXCR4 expressing glioma cells (88). This growth enhancing ability can be explained by downstream induction of phosphorylation and activation of extracellular signal-regulated kinases 1/2 and Akt (89). Studies performed with acute or chronic lymphoproliferative disorders have shown that CXCR4 is up-regulated in many B and T cell malignancies (90, 91). Cutaneous Tlymphoma cells express a functionally active CXCR4 and CXCL12 is abundantly produced in the skin, which represents the main destination of lymphoma cell spreading (92). Furthermore, anti-CXCR4 antibodies abrogated tumour growth in the majority of non-Hodgkin's lymphoma xenograft bearing mice (93). Recent studies show that CXCR4 is essential for homing and maintenance of HSC in distinct stromal cell niches within the marrow (94). Chemotactic responsiveness of hematopoietic stem cells is restricted to CXCL12, which is constitutively secreted by marrow stromal cells. CXCR4 expression levels have a major prognostic impact in acute myeloid leukaemia. There is growing *in vitro* and *in vivo* evidence that CXCR4 expression by leukaemia cells allows for homing and retention within the marrow. Thus, leukaemia cells appear to utilize CXCR4 to access niches that are normally restricted to progenitor cells, and thereby reside in a microenvironment that favors their growth and survival (94).

For a long time CXCR4 was thought to be the sole receptor for CXCL12. However, an additional CXCL12- binding chemokine receptor, CXCR7, was identified (95). Despite its high affinity for CXCL12, the role of CXCR7 in chemotaxis is still a matter of debate. Although one study claims that CXCR7 is involved in chemotaxis (96), more recent data indicate that CXCR7 lacks intrinsic chemotactic activity towards its ligand CXCL12 (97-99). Since the disclosure of CXCR4 as a co-receptor for human immunodeficiency virus (HIV), various CXCR4 antagonists have been made, including the Horseshoe crab protein polyphemusin II and its analogues (100-103) the small molecule heterocyclic bicyclam AMD3100 (104) and the monocyclam AMD3465 (105). AMD3100 was originally developed as a CXCR4 inhibitor with anti-HIV-1 activity but was withdrawn from phase 2 clinical trial primarily because of lack of antiviral effect and the occurrence of unexplained cardiotoxicity. AMD3100 development was continued for hematopoietic stem cell mobilization for which it is recently approved by the FDA to enhance mobilization of stem cells for autologous transplantation in non-Hodgkin lymphoma and multiple myeloma patients. In addition it is studied preclinically for its role as anticancer agent and for the treatment of autoimmune disease (see Table **3**).

3.3.2. CXCR3- CXCL9, CXCL10, CXCL11 & CXCR3B - CXCL4

The chemokine receptor, CXCR3 has three ligands namely CXCL9, CXCL10, and CXCL11. It is expressed in various haematological (106) and solid cancers (107, 108) including prostate (109) and renal cell carcinoma (RCC) (110). Moreover in RCC patients, high CXCR3 expression in the tumours was an independent predictor of improved disease-free survival (DSF) following nephrectomy for localized disease (110). CXCR3 expression in human colon carcinoma cell lines has been associated with tumour cell migration, with CXCL10 being found in the preferred metastatic sites of colorectal cancer (CRC) (111). Several CXCR3 ligands are chemoattractants for malignant B-cells (112), melanoma cells (113), and mucosa associated lymphoid tissue (MALT) cells (114). In general the CXCR3/CXCR3 ligand axis plays an important role in mediating type 1 cytokine-dependent cellmediated immunity. In a murine RCC model, administration of systemic IL-2 induced CXCR3 expression on circulating mononuclear cells. However, IL-2 impaired the CXCR3 ligand chemotactic gradient from plasma to tumour by increasing CXCR3 ligand plasma levels. The antitumour effect of systemic IL-2 was CXCR3-dependent, as IL-2 failed to inhibit tumour growth and angiogenesis in CXCR3-/- mice. Mice treated with a combination of systemic IL-2 and an intratumourally injected CXCR3 ligand (CXCL9) showed greater reduction in tumour growth and angiogenesis, increased tumour necrosis, and increased tumour infiltration of CXCR3+ mononuclear cells, compared with either IL-2 or CXCL9 alone (115). So a combined strategy of systemic IL- 2 with intratumourally injected CXCR3 ligand is probably more efficacious than either strategy alone for reduction of tumour-associated angiogenesis and augmenting tumourassociated immunity, the concept of immunoangiostasis. In 20 metastatic clear cell RCC patients the kinetics of CXCR3 expression in circulating mononuclear cells and expression of all its ligands in plasma has been evaluated during high dose IL-2 therapy (116). Two patients had a complete tumour response. At baseline, no differences were present between CXCR3 expression in peripheral blood mononuclear cells (PBMC) in these RCC patients versus healthy controls. During treatment, PBMC CXCR3 expression increased, as did its ligands. CXCR3 expression rose in each subset, CD4, CD8, and NK cells, in response to high dose IL-2. Although this trend is seen in all 20 patients receiving treatment, the extent of CXCR3 expression varied between subjects, reflecting the variability in responses to high dose IL-2 therapy. The induction of CXCR3 expression by PBMC was most pronounced in a patient who experienced a complete tumour response. Therefore, the authors conclude that the CXCR3/ CXCR3 ligand biologic axis may be an important biomarker in clear cell RCC patients who are undergoing high dose IL-2 therapy (116). CXCL4, also known as platelet factor 4, is released from alpha-granules of activated platelets and has a high binding affinity for CXCR3-B, a splice variant of CXCR3 (117, 118). CXCR3-B is an independent factor for extensive tumour necrosis pattern in RCC (119). Changes in CXCL4 were detected in early growth of human liposarcoma, mammary adenocarcinoma, and osteosarcoma in mice. Moreover, CXCL4 was able to predict the presence of a microscopic, nonangiogenic (dormant) human tumour in mice and circulates predominantly in platelets early in the disease process (120). It is hypothesized that CXCL4 expression counterbalances vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) signaling pathways and therefore keeps tumours in a dormant stage by downregulating angiogenesis (121, 122). Suyama *et al*. postulated that up-regulated CXCR3 ligands in RCC tissue,

produced by tumour vessels, may interact with CXCR3 expressed in tumour cells, with migratory activities leading to RCC progression. In RCCs, CXCL9, CXCL11 and their receptor, CXCR3, are expressed (123). CXCR3 is expressed by tumour cells and infiltrating leukocytes. Furthermore, CXCR3 ligands expressions are much higher both at the mRNA and the protein level than in normal kidney tissue. CXCL11 is present in pericytes and vascular smooth muscle cells in tumour vessels, CXCL9 in tumour endothelial cells and in infiltrating leukocytes. In CRC patients CXCL10 expression was found in the primary tumour and in the preferred metastatic sites of CRC. CXCL10 up-regulates the secretion of matrix metalloproteinase-9 (MMP9) by CRC cells and promotes the adhesion of (metastatic) CRC cells to laminin *in vitro*. Migration in response to CXCL10 could *in vitro* only be shown for the cell lines derived from the metastases. Therefore it is suggested that CXCL10 has an interstitial motility and adherence activity in CRC cells that have reached their metastatic loci (111). Inhibition of CXCR3 expression in a murine melanoma model resulted in fewer metastases to lymph nodes. This lowered CXCR3 expression was accomplished by construction of B16F10 cells transduced with a CXCR3 antisense RNA vector. Thereafter the metastatic potential of these cells after subcutaneously inoculations in mice was studied and compared with mice inoculated with the empty vectortransduced cells. Additional pretreatment of mice with complete Freund's adjuvant increases the levels of CXCL9 and CXCL10 in the draining lymph nodes, which causes a pronounced increase in metastatic frequency of B16F10 cells to the lymph nodes with much larger foci. The specificity of this finding was underscored by the following findings. This stimulation of lymph nodes metastasis by the complete Freund's adjuvant preinjection was significantly lower in the CXCR3 antisense transfectant inoculated clones or when the mice were treated with specific CXCL9 and CXCL10 antibodies (113). In a murine metastatic mammary cancer model, systemic administration of AMG487, a potent and selective orally bioavailable CXCR3 antagonist, inhibited development of lung metastases while local tumour growth was not affected (108). A total of nine healthy Caucasian male volunteers were enrolled into a randomized, double-blind, multipledose, two-period crossover study to evaluate the pharmacokinetics after multiple oral dosing of AMG 487 (124). AMG 487 exhibited linear pharmacokinetics on both day 1 and 7 at the 25-mg dose with dose- and time-dependent kinetics at the two higher doses.

3.3.3. CCR5- CCL3, CCL3L1, CCL4, CCL5, CCL6, CCL8, CCL12

CCR5 is expressed both in haematologic malignancies (125-128) and in solid tumours (129-131), and is associated with cancer metastasis (128, 129, 131, 132) and poor prognosis in gastric cancer. Following gastrectomy, patients with CCR5 expression in their tumour had a 5-year survival rate of 54%, compared to 19% in patients without this CCR5 expression (133). Furthermore, a 10-year disease free of 75% was observed in primary breast cancer patients with CCR5 expression in their tumour as compared to 55% in those without functional CCR5 expression (134). *In vitro,* CCL5 secretion by Reed–Sternberg and Hodgkin lymphoma cells (together H-RS) induces a chemotactic response of human CD4+ T lymphocytes and eosinophils. The production of CCL5 by H-RS cells, leads to autocrine signaling plus the possible production of CCL5 by T-cells. This can lead to more stimulation of the CCR5 on H-RS cells and therefore can contribute to H-RS cell proliferation. Furthermore, anti-CCL5 monoclonal antibodies inhibit *in vitro* basal proliferation of Hodgkin lymphoma-derived cell lines and recombinant CCR5 ligands increase their clonogenic growth (135). In a patient with CRC an accumulation of CCR5 expressing T cells was detected along

the invasive margin of the primary tumour. Its ligand CCL5, which is also secreted by activated platelets, was localized within the infiltrating CD8+ T cells (130, 136). Earlier studies showed that the number of intraepithelial CD8+ T cells in CRC was an independent favorable prognostic factor for patient survival. The T-cells infiltrating the tumour may be involved in antitumour immunity and thus lead to survival advantage. The CCL5, released by the infiltrating T-cells is possibly directly involved in anti-tumour immunity by enhancing the levels of antigen-specific Th1- and cytotoxic T-lymphocyte responses, by upregulating the lysis of target cells, and costimulatory functions of antigen presenting cells (137, 138). Several CCR5 ligands interact with the tumour microenvironment. Human breast cancer cell-derived CCL5 promotes macrophage migration to tumour sites and MMP-9 production by tumour-associated macrophages (139, 140). In a RCC murine model CCL3 was detected in infiltrating cells and in tumour cells, together with an infiltration of leukocytes expressing CCR5. In CCL3 or CCR5 gene deficient mice there are markedly less metastatic foci in the lung, and analysis revealed that both bone marrow- and nonbone marrow-derived cells contributed to metastasis formation. In accordance with these results CCL3- and CCR5-deficient mice showed a reduction in intratumoural accumulation of macrophages, granulocytes, and fibroblasts (141). The same authors demonstrate that CCR5 expression by pulmonary mesenchymal cells promotes migration of mesenchymal cells to CCL4 *in vitro* and increases pulmonary metastasis *in vivo* in a murine melanoma model by induction of MMP9 in the host lung (132, 142). This leads to the conclusion that the CCL3-CCR5 axis regulates intratumoural trafficking of MMP-9-expressing leukocytes and hepatocyte growth factor-expressing fibroblasts, thereby increasing the incidence of metastatic foci in the lung of mice. CCR5 antagonist TAK-779 inhibited cell proliferation and migration of a prostate cancer cell line *in vitro* (131), and neutralizing anti-CCL5 monoclonal antibodies inhibited in Hodgkin lymphoma-derived cell lines basal proliferation (135). Human pancreatic adenocarcinoma tumours produce increased levels of CCL3-5 compared to normal pancreatic tissue. Tregs (CD4+CD25+Foxp3+regulatory T cells) found within this tumour microenvironment express CCR5. These Tregs are important because tumours evade immune destruction by actively inducing immune tolerance by the recruitment of Treg. Reducing CCL5 levels with shRNA (by transducing a tumour cell line with a lentiviral vector producing short hairpin (sh)RNA), and inhibiting CCR5 signaling with systemic administration of TAK-770 resulted in a murine pancreatic adenocarcinoma model in disruption of CCR5- dependent chemotaxis and decreased migration of Treg to tumours and eventually to smaller tumours than in control mice (143). CCR5 is the co-receptor for the most commonly transmitted HIV-1 strains (144). Various drugs have been developed to target the CCR5 receptor including maraviroc (145), E-913 (146), SCH-C (147), TAK-779 (148), and vicriviroc (149). A recent study describes an allogeneic stem-cell transplantation in a HIV infected patient with acute myeloid leukaemia. As transplant served stem cells from an HLAmatched, unrelated donor who was screened for homozygosity for the *CCR5* delta32 deletion. The patient experienced a complete remission of the AML, and the patient's peripheral blood monocytes changed from a heterozygous to a homozygous genotype for *CCR5* delta32 allele. The highly active anti-retroviral therapy (HAART) was discontinued for more than 20 months, and HIV-1 virus RNA or proviral DNA could not be detected in peripheral blood, bone marrow, or rectal mucosa (150). This illustrates that CCR5 plays a prominent role in HIV infection and possibly in AML progression.

3.3.4. CCR7- CCL19, CCL21

CCR7 has as ligands CCL19 and CCL21. Expression of CCR7 is an independent prognostic factor for metastatic non small lung cancer (151) and gastric carcinoma (152) and associated with shorter overall survival (OS) in cervical cancer (153). Cancer cells may well exploit similar mechanisms to access the lymphatics as compared to T-cells. CCR7 is known for its role in homing of T cells to the lymph nodes (154). One of the CCR7 ligands, CCL21, is expressed in the high endothelial venules of lymph nodes, and is necessary for homing of CCR7 positive T cells into the lymph nodes (155). Indeed, the incidence of lymph node metastases correlated with the presence of CCR7 on tissue sections of human cancers including breast cancer (156) and melanoma (157, 158) as well as CRC (159), head and neck (160), prostate (161), non-small lung (151), esophageal squamous cell (162), and gastric (152, 163) cancers. Melanoma cell lines express more CCR7 than normal primary melanocytes (81). There is a strong correlation between CCR7 mRNA expression in melanoma cell lines and CCL21 induced cell migration. Furthermore, CCR7 expression in primary melanomas correlated positively with Breslow thickness. CCL21 mRNA expression measured in sentinel lymph nodes biopsies of 55 melanoma patients correlated inversely with Breslow thickness. The authors conclude that enhanced CCL21 expression in tumour-draining lymph nodes could selectively recruit CCR7 positive metastatic melanoma cells (157). In lymph node samples of breast cancer patients, both CCR7 ligands CCL19 and CCL21 show abundant expression. CCL21 exhibits peak levels of expression in organs representing the first destinations of breast cancer metastases like liver, lung and bone tissue. In breast cancer cells, signaling through CCR7 mediated actin polymerization and pseudopodia formation, which leads to chemotactic and invasive responses of the CCR7 expressing cells towards ligands expressing organs. In 63% of patients who underwent a resection for non small cell lung cancer mRNA of CCR7 was present in the tumour (151). In contrast, in none of the adjacent normal lung tissues CCR7 expression was detected. CCR7 mRNA expression is associated with lymph node metastasis, tumour stage, lymphatic invasion, CCR7 protein and CXCR4 protein expression. Moreover, multivariate analysis showed that CCR7 mRNA expression is an independent predictor of lymph node metastasis. A correlation between expression of CCR7 and lymph node metastasis was also found in cervical cancer (153). In normal cervical tissues, no CCR7 was expressed. In tumour specimen of 174 patients with the International Federation of Gynecology and Obstetrics (FIGO) stages IB–IIB cervical cancers, 59% stained positive for CCR7. The CCR7 expression is higher in squamous cell carcinomas as compared to the adenocarcinomas. Furthermore, CCR7 is increased in larger tumours, in case of deep stromal invasion, vaginal invasion, lymph–vascular space involvement, and in tumours from patients with lymph node metastasis. Multivariate analysis revealed that CCR7 expression is, like in non small lung cancer, an independent predictive factor for pelvic lymph node metastasis. DFS and OS rates of patients with enhanced CCR7 expression (estimated 5-years DFS 79.4%, OS 81.8%) are lower as compared to patients demonstrating no CCR7 expression (estimated 5-year DFS 95.4%, OS 96.1%). Like in cervical cancer, expression of both CXCR4 and CCR7 is an independent unfavorable prognostic factor for OS in patients with esophageal squamous carcinoma (162). In these patients, treated with curative surgery, high CCR7 tumour expression correlated with presence of lymph node metastasis, tumour depth, stage of disease and poor survival, with estimated 5-years OS rates

of 35% for patients with high CCR7 and 70 % for those with low CCR7 expression. In an elegant study by Shield *et al*. a mechanism for CCR7-mediated tumour cell chemotaxis to lymphatics was elucidated. They showed that tumour cells, in addition to sensing chemotactic gradients of CCL21/19 in lymphatics, also generate autologous gradients of CCR7 ligands by secreting the ligands into the ECM under the influence of slow interstitial flow. This mechanism uses the drainage function of lymphatics to direct tumour cells in a chemotactic manner toward lymphatic vessels serving the tumour, thus promoting tumour cell migration towards functional lymphatics (164). Human CCR7 mAb are used *in vitro* to block migration of human CLL cells in response to CCL19. Moreover the CCR7 antibodies mediate a potent, complement dependent cytotoxicity against CLL cells while sparing normal T lymphocytes from the same patient (165). In a metastatic CCR7 cDNA transduced melanoma mouse model the expression of CCR7 promotes the metastasis of melanoma cells to regional lymph nodes. This process is completely blocked by neutralizing anti-CCL21 antibodies but is not affected by control IgG (158). These results suggest that blocking CCR7 plays an important role in prevention of metastasis.

3.3.5. CXCR1 – CXCL6, CXCL7, CXCL8 & CXCR2 – CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8

CXCR1 and 2 are expressed in glioblastoma multiforme (GBM) (166), in prostate cancers (167), and in RCC (CXCR2) (168). In breast cancer cell lines CXCR2 expression is lower compared to the tissue of origin (81). Various studies demonstrate the expression of CXCR1/2 ligands. CXCL8 was found in 38% of GBMs (166), in prostate cancers (167), head and neck squamous cell carcinomas (169), and non small cell lung cancers (170-172). In the last tumour type CXCL5 was also present. In addition to CXCL8 and CXCL5, CXCL1 and CXCL3 were detected in tumours and plasma from patients with metastatic RCC (168). CXCL6 was expressed in small cell lung cancer tissue and in non small cell lung cancer cell lines (173). The expression of CXCL8, which is together with CXCL1, CXCL5 and CXCL7 also secreted by activated platelets (136), is directly correlated with poor survival in non small cell lung cancer (174-178) and ovarian cancer (179). Moreover, elevated CXCL8 expression level is positively associated with cancer progression in prostate cancer (180, 181), bladder cancer (182), ovarian cancer (183) and melanoma patients (184, 185). The aggressiveness of malignant melanoma is attributed, in part, to the expression of CXCL8 and its receptors CXCR1 and CXCR2. Analysis of CXCR2 and CXCL8 in human melanomas shows that CXCR2 is expressed predominantly by higher grade melanomas and melanoma metastases (186). Enhanced CXCR2 expression is also correlated with tumour progression in GBM (166). In experimental models of human non small cell lung cancer tumourigenesis, CXCL5 expression is directly correlated with tumour growth, tumour-derived angiogenesis, and metastatic potential (173). Furthermore, CXCL8 correlates with the angiogenesis, tumourigenicity, and metastatic potential of many solid cancers in xenograft and orthotopic *in vivo* models (181, 187-190). CXCL8 secreted by tumour cells, induces the differentiation and activation of osteoclasts, supporting the finding of the characteristic osteolytic metastasis of breast cancer cells that have disseminated to the bone (191). Moreover, in CXCR2 knockout mice with metastatic RCC the potential to metastasize to the lungs is decreased (168). Cancer stem cells (CSC) express CXCL8. In various breast cancer cell lines (BrCa-MZ-01, MDA-MB-453, SUM159, MCF7, S68) qRT-PCR showed an increase of CXCR1 in cancer cells which are displaying stem cell properties. Furthermore, treating four breast cancer

cell lines with recombinant human CXCL8 did increase the formation of primary and secondary tumour spheres in a dose-dependent manner. This means that CXCL8 promotes CSC self-renewal (192). Interestingly, CXCL8 expression and secretion can be induced by chemotherapeutics such as 5-fluorouracil, doxorubicin, dacarbazine, paclitaxel in various cancer cell lines (193-195). Moreover chemotherapeutic agents also induce transcriptional regulation of CXCR1/2 genes and increase the level of CXCL8 signaling by the prostate cancer cell lines (196). By using liposome-encapsulated small interfering RNA (siRNA) as a treatment within ovarian tumour xenografts to suppress CXCL8 expression Merritt *et al.* showed growth retardation, reduced microvessel density, and an increased response to the docetaxel (197, 198). Several successful treatment interventions have been described. Many small molecule inhibitors of CXCR1/2 signaling with appropriate pharmacokinetic properties, and therefore suitable for preclinical animal models, are now emerging (199-204). Treatment of nude mice with ABX-CXCL8, a human IgG2 monoclonal antibody neutralizing human CXCL8 signaling, reduces growth of tumours in nude mice injected with human melanoma cell lines (187). Anti-CXCL8 neutralizing antibody also decreases cell proliferation in two non small cell lung cancer cell lines H460 and MOR/P (205). In SCID mice anti-CXCL1 antibodies inhibit the tumourigenesis and tumour-related angiogenesis of a CXCL1- producing prostate cancer cell line, Du145 (206).

3.3.6. Other Chemokine Receptors and their Ligands

CCR1 - CCL3, CCL3L1, CCL5, CCL9/CCL10, CCL14-16

CCR2 - CCL2, CCL7-8, CCL12-13

CCR3 - CCL5, 6, CCL11, CCL15-16, CCL23-24, CCL26

CCR4 - CCL17, CCL22, CCR6 - CCL20, CCR8 - CCL1

CCR9 - CCL25, CCR10 - CCL27-28, XCR1- XCL1-2

CX3CR1 - CX3CL1, CXCR5 - CXCL13, CXCR6 - CXCL16

CXCR4 and CCR7 are likely to be the most important chemokine receptors in many cancers, while other chemokine receptors may participate in the metastasizing of specific cancers. For example, CCR3 is expressed in CD30+ cutaneous lymphomas, and its ligand CCL11 is often expressed in tumour-associated skin lesions (206, 208). In peripheral blood samples of patients with CLL, B cells express high levels of functional CXCR5. Stimulation of CLL cells with CXCL13 induces actin polymerization, CXCR5 endocytosis, chemotaxis, and prolonged activation of p44/42 MAPK. Anti-CXCR5 antibodies, pertussis toxin, and wortmannin (a fungal metabolite obtained from Penicillium fumiculosum that specifically inhibits PI-3K, MAPK, and myosin lightchain kinase), inhibit chemotaxis to CXCL13, demonstrating the importance of Gi proteins and PI3 kinases for CXCR5 signaling (75, 209). Purified follicular lymphoma cells, express CX3CR1 in contrast to non-malignant B cell populations (210). CXCR5 was found to be expressed in 21 of 24 gliomas, while its ligand CXCL13 was only shown in 4 of 25 samples. The expression of the ligands occurred more frequently in GBM (WHO grade IV) then in lower grades astrocytomas (WHO grade I-III), which indicates a role for CXCR5 in glioma progression (166). The expression of chemokine receptors in cancer cells mirrors the expression of these receptors in their normal counterparts. For example,

CCR8 mRNA expression can be found both in normal breast epithelial cells and in breast cancer cells, but to a higher level in the tumour cells (81). Also, CXCL13 is overexpressed in breast cancer cell lines, in primary breast cancers and in the peripheral blood of breast cancer patients. Moreover, serum CXCL13 levels in patients with metastatic disease are elevated as compared with controls and disease-free patients (211). In contrast to the CXCR5 ligand CXCL13, levels of CXCR5 are low in malignant and healthy breast tissues, and surface expression could not be detected *in vitro* (211). CXCR6 en CXCL16 were elucidated in all of 170 human primary breast carcinomas and at similar levels in all of 8 human breast cancer cell lines (212). Surprisingly, suppression of CXCR6 as well as CXCL16 leads to enhanced proliferation *in vitro* as well as *in vivo*, indicating that their interaction inhibits proliferation. It is remarkable that both CXCR6 and CXCL16 are expressed by all breast cancers, because one would expect that cells that loose either one acquire a growth advantage and would be expected to be selected during tumour progression. This suggests an unknown important role for the CXCR6/CXCL16 axis in tumour formation. Proteases, possibly macrophage derived, might convert inhibitory transmembrane CXCL16 into the stimulatory chemokine. A complex chemokine/chemokine receptor network in ovarian cancer ascites was reported by Milliken *et al*. (213). In cell isolates of this ascites mRNA for the CC chemokines CCL2, -3, -4, -5, -8, and -22 and for CC chemokine receptors CCR1, -2a, -2b, -3, -4, -5, and -8 was detected. The corresponding proteins were also detected in the ascitic fluid. However, no correlation between chemokine/chemokine receptor levels and infiltrating leukocytes could be detected. Of all these chemokines both CCL3 and CCL7 are also secreted by activated platelets (136). Monocyte chemoattractant protein 1 (CCL2) is a prominent regulator of prostate cancer growth and metastasis. In an animal model using VCaP cells, anti-CCL2 treatment suppresses tumour growth in bone. The decreased tumour burden in the bone is associated with less bone resorption and microvessel density in the bone lesions was reduced by 70% in anti-CCL2-treated animals with bone lesions (214). These data suggest that a destructive cascade is driven by tumour cell-derived, induction of CCL2, which facilitates tumour growth via enhanced osteoclastic and endothelial cell activity in bone marrow. Taken together, CCL2 mediates the interaction between tumour-derived factors and host-derived chemokines acting in cooperation to promote skeletal metastasis. CX3CR1 is present in human prostate cancer cell lines, whereas human bone marrow endothelial cells and differentiated osteoblasts expressed its ligand CX3CL1. The adhesion of prostate cancer cells to human bone marrow endothelial cells in flow conditions can be reduced by a neutralizing CX3CL1 antibody (215). CCR6 and CCL20 expression were studied in 80 prostate cancers of various grades and stages. Expression levels of CCR6 were associated with clinical and pathologic features of more advanced and aggressive prostate cancer (216). Enhanced expression of CXCL16 and CXCR6 was notified in primary RCC tissue and in RCC cell lines (217). High levels of CXCL16 expression in RCCs correlate with better survival of patients, and CXCL16 correlates inversely with tumour stage. *In vitro* inhibition of CXCL16 in RCC, achieved by siRNA constructs and with anti-CXCL16 antibodies, increases the migration of RCC cells (217). In esophageal carcinoma, CCL2 expression is positively correlated with the level of macrophage infiltration, tumour angiogenesis, and depth of tumour invasion (218). These results indicate that tumour-infiltrating macrophages may facilitate tumour growth and progression. CCL2 is secreted by neoplastic ducts from surgical pancreatic carcinoma

samples. In an *in vitro* pancreatic carcinoma model inflammatory cytokines such as IFNγ, TNFα, and IL-1β increase CCL2 expression. After resection of the tumour patients with high serum CCL2 levels have a better survival compared to patients with lower CCL2 levels. Serum CCL2 levels are positively correlated with intratumoural macrophage infiltration and inversely correlated with tumour cell proliferative activity. Interestingly, pancreatic cancer cells do not express functional receptors for CCL2 (208, 219). CCL11 and CCL2 expression was higher in biopsies of resected colorectal hepatic metastases as compared to nonneoplastic adjacent liver. Immunohistochemical staining indicates that carcinoembryonic antigen-positive tumour cells stain strongly for CCL11. *In vitro* studies confirmed that several CRC lines produce CCL11. Jurkat T-cells, engineered to express the receptor for CCL11 (CCR3), effectively migrate in response to CCL11 protein (220). In addition CCR6 expression in primary CRC is independently associated with the presence of liver metastases (221). Finally, some chemokine receptors have never been found in tumour cells, including receptors such as XCR1, which have a very restricted expression (75).

3.3.7. Formylated Peptide Receptor

The formylated peptide receptor (FPR) also belongs to the family of GPC seven-transmembrane receptors and was originally identified in phagocytic leukocytes, which mediated cell chemotaxis and activation in response to the bacterial chemotactic peptide fMLP. The capacity of growth and invasion of astrocytomas is correlated with the expression of cell surface receptors that sense the signals present in the tumour microenvironment. High grade human astrocytoma cells express functional FPR and by responding to potential agonist(s) released by necrotic tumour cells, FPR promotes the directional migration, survival, and production of angiogenic VEGF by tumour cells (222, 223). FPR is a GPCR, originally identified in phagocytic leukocytes, which mediates cell chemotaxis and activation in response to the bacterial chemotactic peptide fMLP. During the past few years, a number of novel and host-derived chemotactic agonists of FPR have been identified in addition to fMLP, including formyl peptides potentially released by mitochondria of ruptured cells (224), annexin I produced by activated epithelia (225), and a neutrophil granule protein, cathepsin G (226). Agonist binding to FPR elicits a cascade of signal transduction pathways that involve PI3K, MAPK, and the transcription factor nuclear factor-B (227), which are linked with tumour-promoting functions like proliferation and migration. Functional FPR has been detected in cells of nonhematopoietic origin, such as lung epithelial cells (228) and hepatocytes (229). Depletion of FPR by siRNA markedly reduces the tumourigenicity of the astrocytoma cells in immunodeficient mice (230). Tumour nodules formed by U-87 cells transfected with FPR siRNA appeared later, and the corresponding tumours grow slower than those formed by wild-type U-87 cells or by mock-transfected cells. By day 42 after implantation, all mice implanted with wild-type or mock-transfected U-87 cells had died or had to be sacrificed because of large necrotic tumours. In contrast, all mice bearing tumours formed by FPR siRNA-transfected U-87 cells survived over 72 days after implantation. fMLP induces EGFR phosphorylation at tyrosine residue (Tyr) 992, but not residues 846, 1068, or 1173, in astrocytoma cells, whereas all these residues were phosphorylated after only EGF treatment. The FPR agonist-induced EGFR phosphorylation in tumour cells is dependent on the presence of FPR as well as Gi proteins, and is controlled by Src tyrosine kinase. The transactivation of EGFR contributes to the biological function of FPR in

astrocytoma cells because inhibition of EGFR phosphorylation significantly reduced FPR agonist-induced tumour cell migration and proliferation. Furthermore, depletion of both FPR and EGFR by short interference RNA abolished the tumourigenesis of the astrocytoma cells. This study indicates that the astrocytomapromoting activity of FPR is mediated in part by transactivation of EGFR and the cross-talk between two receptors exacerbates the malignant phenotype of tumour cells and targeting both receptors may yield superior therapeutic effects compared with targeting either one receptor.

4. CONCLUSIONS

Chemokines are small cytokines that have the ability to induce migration of cells expressing chemokine receptors. When chemokines bind their GPCR downstream signaling induces actin polymerization and eventually cell shape change and chemotaxis. The important role of these chemotactic cytokines starts in embryonic development. During infection chemokines induce migration of leukocytes and activate integrins on the leukocyte surface. The last decade studies provide evidence for an important role of chemokines in the oncology field. Chemokines and their receptors are influencing the growth of primary tumours and the development of metastases and expression was sometimes found to be directly correlated to survival of patients. The mechanisms by which the different chemokines and their receptors modulate tumour cell behaviour are cell proliferation, angiogenesis, local invasion and metastasis. Chemokines do not only induce receptor signaling but also interact with the microenvironment of tumours. Manipulation of chemokines and their receptors could become important to incorporate into new anticancer strategies**.**

REFERENCES

1. Zlotnik A. Chemokines and cancer. Int J Cancer 2006;119:2026- 2029.
2. Takeuchi H, Kitago M, Hoon DS. Effects of chemokines on tumour metastasis. Cancer Treat Res 2007;135:177-184.
3. Smith MC, Luker KE, Garbow JR, *et al*. CXCR4 regulates growth of both primary and metastatic breast cancer. Cancer Res 2004;64:8604-8612.
4. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832-1837.
5. Palczewski K. G protein-coupled receptor rhodopsin. Annu Rev Biochem 2006;75: 743-767.
6. Sabirsh A, Bywater RP, Bristulf J, Owman C, Haeggstrom JZ. Residues from transmembrane helices 3 and 5 participate in leukotriene B4 binding to BLT1. Biochemistry 2006;45:5733-5744.
7. Mills JS, Miettinen HM, Cummings D, Jesaitis AJ. Characterization of the binding site on the formyl peptide receptor using three receptor mutants and analogs of Met-Leu-Phe and Met-Met- Trp-Leu-Leu. J Biol Chem 2000;275:39012-39017.
8. Ishii I, Izumi T, Tsukamoto H, Umeyama H, Ui M, Shimizu T. Alanine exchanges of polar amino acids in the transmembrane domains of a platelet-activating factor receptor generate both constitutively active and inactive mutants. J Biol Chem 1997;272:7846-7854.
9. Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. Annu Rev Immunol 2007;25:787- 820.
10. Siciliano SJ, Rollins TE, DeMartino J, *et al*. Two-site binding of C5a by its receptor: an alternative binding paradigm for G protein-coupled receptors. Proc Natl Acad Sci USA 1994;91:1214-1218.
11. Fernandez EJ, Lolis E. Structure, function, and inhibition of chemokines. Annu Rev Pharmacol Toxicol 2002;42:469-499.
12. Hamm HE. The many faces of G protein signaling. J Biol Chem 1998;273:669-672.
13. Dominguez F, Pellicer A, Simon C. Paracrine dialogue in implantation. Mol Cell Endocrinol 2002;186:175-181.
14. Ozenci CC, Korgun ET, Demir R. Immunohistochemical detection of CD45+, CD56+, and CD14+ cells in human decidua during early pregnancy. Early Pregnancy 2001;5:164-175.
15. Moffett-King A, Entrican G, Ellis S, Hutchinson J, Bainbridge D. Natural killer cells and reproduction. Trends Immunol 2002;23: 332-333.
16. Trundley A, Moffett A. Human uterine leukocytes and pregnancy. Tissue Antigens 2004;63:1-12.
17. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol 2002;2:656-663.
18. Croy BA, Esadeg S, Chantakru S, *et al*. Update on pathways regulating the activation of uterine Natural Killer cells, their interactions with decidual spiral arteries and homing of their precursors to the uterus. J Reprod Immunol 2003;59:175-191.
19. Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA. Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. J Clin Endocrinol Metab 2004;89:6155-6167.
20. Robertson MJ, Williams BT, Christopherson K, Brahmi Z, Hromas R. Regulation of human natural killer cell migration and proliferation by the exodus subfamily of CC chemokines. Cell Immunol 2000;199:8-14.
21. Kitaya K, Nakayama T, Okubo T, Kuroboshi H, Fushiki S, Honjo H. Expression of macrophage inflammatory protein-1beta in human endometrium: its role in endometrial recruitment of natural killer cells. J Clin Endocrinol Metab 2003;88:1809-1814.

22. Drake PM, Red-Horse K, Fisher SJ. Reciprocal chemokine receptor and ligand expression in the human placenta: implications for cytotrophoblast differentiation. Dev Dyn 2004;229:877-885.
23. Red-Horse K, Drake PM, Gunn MD, Fisher SJ. Chemokine ligand and receptor expression in the pregnant uterus: reciprocal patterns in complementary cell subsets suggest functional roles. Am J Pathol 2001;159: 2199-2213.
24. Dominguez F, Pellicer A, Simon C. The chemokine connection: hormonal and embryonic regulation at the human maternalembryonic interface--a review. Placenta 2003;24 (Suppl B):S48- S55.
25. Drake PM, Gunn MD, Charo IF, *et al*. Human placental cytotrophoblasts attract monocytes and CD56(bright) natural killer cells via the actions of monocyte inflammatory protein 1alpha. J Exp Med 2001;193:1199-1212.
26. Dominguez F, Galan A, Martin JJ, Remohi J, Pellicer A, Simon C. Hormonal and embryonic regulation of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in the human endometrium and the human blastocyst. Mol Hum Reprod 2003;9:189-198.
27. Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S. Trophoblasts acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype. Development (Cambridge, England) 2003;130:5519-5532.
28. Salamonsen LA, Hannan NJ, Dimitriadis E. Cytokines and chemokines during human embryo implantation: roles in implantation and early placentation. Semin Reprod Med 2007;25:437-444.
29. Ratajczak MZ, Zuba-Surma E, Kucia M, Reca R, Wojakowski W, Ratajczak J. The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumourigenesis. Leukaemia 2006;20: 1915-1924.
30. Nagasawa T, Hirota S, Tachibana K, *et al*. Defects of B-cell lymphopoiesis and bonemarrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635-638.
31. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595-599.
32. Petit I, Szyper-Kravitz M, Nagler A, *et al*. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 2002;3: 687-694.
33. Broxmeyer HE, Cooper S, Kohli L, *et al*. Transgenic expression of stromal cell-derived factor-1/CXC chemokine ligand 12 enhances myeloid progenitor cell survival/ antiapoptosis *in vitro* in response to growth factor withdrawal and enhances myelopoiesis *in vivo*. J Immunol 2003;170:421-429.
34. Broxmeyer HE, Kohli L, Kim CH, *et al*. Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and Gαi proteins and enhances engraftment of competitive, repopulating stem cells. J Leukoc Biol 2003;73:630-638.
35. Broxmeyer HE, Sherry B, Cooper S, *et al*. Comparative analysis of the human macrophage inflammatory protein family of cytokines (chemokines) on proliferation of human myeloid progenitor cells. Interacting effects involving suppression, synergistic suppression, and blocking of suppression. J Immunol 1993;150:3448-3458.
36. McGrath KE, Koniski AD, Maltby KM, McGann JK, Palis J. Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. Dev Biol 1999;213:442-456.
37. Bagri A, Gurney T, He X, *et al*. The chemokine SDF1 regulates migration of dentate granule cells. Development (Cambridge, England) 2002;129:4249-4260.

38. Lu M, Grove EA, Miller RJ. Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. Proc Natl Acad Sci USA 2002;99:7090-7095.
39. Ma Q, Jones D, Borghesani PR, *et al*. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1- deficient mice. Proc Natl Acad Sci USA 1998;95:9448-9453.
40. Reiss K, Mentlein R, Sievers J, Hartmann D. Stromal cell-derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granular layer. Neuroscience 2002;115:295-305.
41. Borrell V, Marin O. Meninges control tangential migration of hemderived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nat Neurosci 2006;9:1284-1293.
42. Stumm RK, Zhou C, Ara T, *et al*. CXCR4 regulates interneuron migration in the developing neocortex. J Neurosci 2003;23:5123-5130.
43. Super H, Del Rio JA, Martinez A, Perez-Sust P, Soriano E. Disruption of neuronal migration and radial glia in the developing cerebral cortex following ablation of Cajal-Retzius cells. Cereb Cortex 2000;10:602-613.
44. D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 1995;374: 719-723.
45. Letinic K, Zoncu R, Rakic P. Origin of GABAergic neurons in the human neocortex. Nature 2002;417:645-649.
46. Uylings HB. Development of the cerebral cortex in rodents and man. Eur J Morphol 2000;38:309-312.
47. de Carlos JA, Lopez-Mascaraque L, Valverde F. Dynamics of cell migration from the lateral ganglionic eminence in the rat. J Neurosci 1996;16:6146-6156.
48. DeDiego I, Smith-Fernandez A, Fairen A. Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. Eur J Neurosci 1994;6:983-997.
49. Anderson SA, Kaznowski CE, Horn C, Rubenstein JL, McConnell SK. Distinct origins of neocortical projection neurons and interneurons *in vivo*. Cereb Cortex 2002;12:702-709.
50. Stumm RK, Rummel J, Junker V, *et al*. A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J Neurosci 2002;22:5865-5878.
51. Tiveron MC, Rossel M, Moepps B, *et al*. Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/ CXCR4 signaling in the cortical subventricular zone/ intermediate zone. J Neurosci 2006;26:13273-13278.
52. Li G, Adesnik H, Li J, *et al*. Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. J Neurosci 2008;28:1085-1098.
53. Sotelo C. Cellular and genetic regulation of the development of the cerebellar system. Prog Neurobiol 2004;72:295-339.
54. Bentley D, O'Connor TP. Cytoskeletal events in growth cone steering. Curr Opin Neurobiol 1994;4:43-48.
55. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. Science 1996;274:1123-1133.
56. Song H, Poo M. The cell biology of neuronal navigation. Nat Cell Biol 2001;3:E81-E88.
57. Xiang Y, Li Y, Zhang Z, *et al*. Nerve growth cone guidance mediated by G protein-coupled receptors. Nat Neurosci 2002;5: 843-848.

58. Arakawa Y, Bito H, Furuyashiki T, *et al*. Control of axon elongation via an SDF-1α/ Rho/ mDia pathway in cultured cerebellar granule neurons. J Cell Biol 2003;161:381-391.

59. Pujol F, Kitabgi P, Boudin H. The chemokine SDF-1 differentially regulates axonal elongation and branching in hippocampal neurons. J Cell Sci 2005;118:1071-1080.

60. Lieberam I, Agalliu D, Nagasawa T, Ericson J, Jessell TM. A CXCL12-CXCR4 chemokine signaling pathway defines the initial trajectory of mammalian motor axons. Neuron 2005;47:667-679.

61. Tran PB, Miller RJ. Chemokine receptors in the brain: a developing story. J Comp Neurol 2003;457:1-6.

62. Ganju RK, Brubaker SA, Meyer J, *et al*. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. J Biol Chem 1998;273: 23169-23175.

63. Edman LC, Mira H, Erices A, *et al*. Alpha-chemokines regulate proliferation, neurogenesis, and dopaminergic differentiation of ventral midbrain precursors and neurospheres. Stem Cells 2008;26: 1891-1900.

64. Tsai HH, Frost E, To V, *et al*. The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. Cell 2002;110:373-383.

65. Tachibana K, Hirota S, Iizasa H, *et al*. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 1998;393:591-594.

66. Panzer U, Steinmetz OM, Stahl RA, Wolf G. Kidney diseases and chemokines. Curr Drug Targets 2006;7:65-80.

67. Grone HJ, Cohen CD, Grone E, *et al*. Spatial and temporally restricted expression of chemokines and chemokine receptors in the developing human kidney. J Am Soc Nephrol 2002;13:957-967.

68. de Haas CJ, Veldkamp KE, Peschel A, *et al*. Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Exp Med 2004;199:687-695.

69. Rooijakkers SH, Ruyken M, Roos A, *et al*. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol 2005;6:920- 927.

70. Jongerius I, Kohl J, Pandey MK, *et al*. Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med 2007;204:2461-2471.

71. Prat C, Bestebroer J, de Haas CJ, van Strijp JA, Van Kessel KP. A new staphylococcal anti-inflammatory protein that antagonizes the formyl peptide receptor-like 1. J Immunol 2006;177:8017-8026.

72. Bestebroer J, Van Kessel KP, Azouagh H, *et al*. Staphylococcal SSL5 inhibits leukocyte activation by chemokines and anaphylatoxins. Blood 2009;113:328-337.

73. Walenkamp AME, Bestebroer J, Boer IGJ, *et al*. Staphylococcal SSL5 binding to human leukaemia cells inhibits cell adhesion to endothelial cells and platelets. Cellular Oncology. 2010;32:1-10.

74. Walenkamp AM, Boer IG, Bestebroer J, *et al*. Staphylococcal superantigen-like 10 inhibits CXCL12-induced human tumour cell migration. Neoplasia 2009;11:333-344.

75. Zlotnik A. Chemokines in neoplastic progression. Semin Cancer Biol 2004;14: 181-185.

76. Zlotnik A. Chemokines and cancer. Int J Cancer 2006;119:2026- 2029.

77. Takeuchi H, Kitago M, Hoon DS. Effects of chemokines on tumour metastasis. Cancer Treat Res 2007;135:177-184.

78. Smith MC, Luker KE, Garbow JR, *et al*. CXCR4 regulates growth of both primary and metastatic breast cancer. Cancer Res 2004;64:8604-8612.

79. Rombouts EJ, Pavic B, Lowenberg B, Ploemacher RE. Relation between CXCR-4 expression, Flt3 mutations, and unfavorable prognosis of adult acute myeloid leukaemia. Blood 2004;104:550- 557.

80. Crazzolara R, Kreczy A, Mann G, *et al*. High expression of the chemokine receptor CXCR4 predicts extramedullary organ infiltration in childhood acute lymphoblastic leukaemia. Br J Haematol 2001;115:545-553.
81. Muller A, Homey B, Soto H, *et al*. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50-56.
82. Zeelenberg IS, Ruuls-Van SL, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Res 2003;63: 3833-3839.
83. Sehgal A, Keener C, Boynton AL, Warrick J, Murphy GP. CXCR- 4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumour cells. J Surg Oncol 1998;69:99-104.
84. Geminder H, Sagi-Assif O, Goldberg L, *et al*. A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. J Immunol 2001;167:4747-4757.
85. Tanabe S, Heesen M, Yoshizawa I, *et al*. Functional expression of the CXC-chemokine receptor- 4/fusin on mouse microglial cells and astrocytes. J Immunol 1997;159: 905-911.
86. Rempel SA, Dudas S, Ge S, Gutierrez JA. Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma. Clin Cancer Res 2000;6:102-111.
87. Ohtani Y, Minami M, Kawaguchi N, *et al*. Expression of stromal cell-derived factor-1 and CXCR4 chemokine receptor mRNAs in cultured rat glial and neuronal cells. Neurosci Lett 1998;249:163-166.
88. Zhou Y, Larsen PH, Hao C, Yong VW. CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. J Biol Chem 2002;277:49481-49487.
89. Barbero S, Bonavia R, Bajetto A, *et al*. Stromal cell-derived factor 1alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. Cancer Res 2003;63:1969- 1974.
90. Pistoia V, Corcione A, Dallegri F, Ottonello L. Lymphoproliferative disorders and chemokines. Curr Drug Targets 2006;7: 81- 90.
91. Zeelenberg IS, Ruuls-Van SL, Roos E. Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a T cell hybridoma. J Clin Invest 2001;108:269-277.
92. Narducci MG, Scala E, Bresin A, *et al*. Skin homing of Sezary cells involves SDF-1-CXCR4 signaling and down-regulation of CD26/dipeptidylpeptidase IV. Blood 2006;107:1108-1115.
93. Bertolini F, Dell'Agnola C, Mancuso P, *et al*. CXCR4 neutralization, a novel therapeutic approach for non-Hodgkin's lymphoma. Cancer Res 2002;62:3106-3112.
94. Burger JA, Burkle A. The CXCR4 chemokine receptor in acute and chronic leukaemia: a marrow homing receptor and potential therapeutic target. Br J Haematol 2007;137:288-296.
95. Burns JM, Summers BC, Wang Y, *et al*. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumour development. J Exp Med 2006;203:2201-2213.
96. Balabanian K, Lagane B, Infantino S, *et al*. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J Biol Chem 2005;280: 35760-35766.
97. Hartmann TN, Grabovsky V, Pasvolsky R, *et al*. A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. J Leukoc Biol 2008;84:1130-1140.

98. Sierro F, Biben C, Martinez-Munoz L, *et al*. Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. Proc Natl Acad Sci U S A 2007;104:14759-14764.

99. Burns JM, Summers BC, Wang Y, *et al*. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumour development. J Exp Med 2006;203:2201-2213.

100. Burger M, Hartmann T, Krome M, *et al*. Small peptide inhibitors of the CXCR4 chemokine receptor (CD184) antagonize the activation, migration, and antiapoptotic responses of CXCL12 in chronic lymphocytic leukaemia B cells. Blood 2005;106:1824-1830.

101. Murakami T, Zhang TY, Koyanagi Y, *et al*. Inhibitory mechanism of the CXCR4 antagonist T22 against human immunodeficiency virus type 1 infection. J Virol 1999;73:7489-7496.

102. Arakaki R, Tamamura H, Premanathan M, *et al*. T134, a small-molecule CXCR4 inhibitor, has no cross-drug resistance with AMD3100, a CXCR4 antagonist with a different structure. J Virol 1999;73:1719-1723.

103. Tamamura H, Xu Y, Hattori T, *et al*. A low-molecular-weight inhibitor against the chemokine receptor CXCR4: a strong anti-HIV peptide T140. Biochem Biophys Res Commun 1998;253:877-882.

104. Hendrix CW, Collier AC, Lederman MM, *et al*. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. J Acquir Immune Defic Syndr 2004;37:1253-1262.

105. Hatse S, Princen K, De CE, *et al*. AMD3465, a monomacrocyclic CXCR4 antagonist and potent HIV entry inhibitor. Biochem Pharmacol 2005;70:752-761.

106. Jones D, Benjamin RJ, Shahsafaei A, Dorfman DM. The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukaemia. Blood 2000;95:627-632.

107. Ohtani H, Jin Z, Takegawa S, Nakayama T, Yoshie O. Abundant expression of CXCL9 (MIG) by stromal cells that include dendritic cells and accumulation of CXCR3+ T cells in lymphocyte-rich gastric carcinoma. J Pathol 2009;217:21-31.

108. Walser TC, Rifat S, Ma X, *et al*. Antagonism of CXCR3 inhibits lung metastasis in a murine model of metastatic breast cancer. Cancer Res 2006;66:7701-7707.

109. Engl T, Relja B, Blumenberg C, *et al*. Prostate tumour CXC-chemokine profile correlates with cell adhesion to endothelium and extracellular matrix. Life Sci 2006;78: 1784-1793.

110. Klatte T, Seligson DB, Leppert JT, *et al*. The chemokine receptor CXCR3 is an independent prognostic factor in patients with localized clear cell renal cell carcinoma. J Urol 2008;179:61-66.

111. Zipin-Roitman A, Meshel T, Sagi-Assif O, *et al*. CXCL10 promotes invasion-related properties in human colorectal carcinoma cells. Cancer Res 2007;67:3396- 3405.

112. Trentin L, Agostini C, Facco M, *et al*. The chemokine receptor CXCR3 is expressed on malignant B cells and mediates chemotaxis. J Clin Invest 1999;104:115-121.

113. Kawada K, Sonoshita M, Sakashita H, *et al*. Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res 2004;64:4010-4017.

114. Suefuji H, Ohshima K, Karube K, *et al*. CXCR3-positive B cells found at elevated frequency in the peripheral blood of patients with MALT lymphoma are attracted by MIG and belong to the lymphoma clone. Int J Cancer 2005;114:896-901.

115. Pan J, Burdick MD, Belperio JA, *et al*. CXCR3/CXCR3 ligand biological axis impairs RENCA tumour growth by a mechanism of immunoangiostasis. J Immunol 2006;176:1456-1464.

116. Reckamp KL, Figlin RA, Moldawer N, *et al*. Expression of CXCR3 on mononuclear cells and CXCR3 ligands in patients with metastatic renal cell carcinoma in response to systemic IL-2 therapy. J Immunother 2007;30:417-424.

117. Eisman R, Surrey S, Ramachandran B, Schwartz E, Poncz M. Structural and functional comparison of the genes for human platelet factor 4 and PF4alt. Blood 1990;76:336-344.

118. Lasagni L, Francalanci M, Annunziato F, *et al*. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP- 10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. J Exp Med 2003;197:1537-1549.

119. Gacci M, Serni S, Lapini A, *et al*. CXCR3-B expression correlates with tumour necrosis extension in renal cell carcinoma. J Urol 2009;181:843-848.

120. Cervi D, Yip TT, Bhattacharya N, *et al*. Platelet-associated PF-4 as a biomarker of early tumour growth. Blood 2008;111: 1201-1207.

121. Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumour metastasis. Proc Natl Acad Sci USA 2001;98:3352-3357.

122. Maione TE, Gray GS, Petro J, *et al*. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 1990;247:77-79.

123. Suyama T, Furuya M, Nishiyama M, *et al*. Up-regulation of the interferon gamma (IFNgamma)- inducible chemokines IFN-inducible T-cell α chemoattractant and monokine induced by IFN-γ and of their receptor CXC receptor 3 in human renal cell carcinoma. Cancer 2005;103:258-267.

124. Tonn GR, Wong SG, Wong SC, *et al*. An inhibitory metabolite leads to dose- and time-dependent pharmacokinetics of (R)-N-{1-[3-(4-ethoxy-phenyl)-4-oxo-3,4-dihydro-pyrido[2,3-d]pyrimidin-2-yl]-ethy l}-N-pyridin-3-ylmethyl- 2-(4-trifluoromethoxy-phenyl)-acetamide (AMG 487) in human subjects after multiple dosing. Drug Metab Dispos 2009;37:502-513.

125. Trentin L, Cabrelle A, Facco M, *et al*. Homeostatic chemokines drive migration of malignant B cells in patients with non-Hodgkin lymphomas. Blood 2004;104: 502-508.

126. Lentzsch S, Gries M, Janz M, Bargou R, Dorken B, Mapara MY. Macrophage inflammatory protein 1-alpha (MIP-1 alpha) triggers migration and signaling cascades mediating survival and proliferation in multiple myeloma (MM) cells. Blood 2003;101:3568- 3573.

127. Maggio EM, Van Den BA, Visser L, *et al*. Common and differential chemokine expression patterns in rs cells of NLP, EBV positive and negative classical Hodgkin lymphomas. Int J Cancer 2002;99:665-672.

128. Makishima H, Ito T, Asano N, *et al*. Significance of chemokine receptor expression in aggressive NK cell leukaemia. Leukaemia 2005;19:1169-1174.

129. Luboshits G, Shina S, Kaplan O, *et al*. Elevated expression of the CC chemokine regulated on activation, normal T cell expressed and secreted (RANTES) in advanced breast carcinoma. Cancer Res 1999;59:4681-4687.

130. Musha H, Ohtani H, Mizoi T, *et al*. Selective infiltration of CCR5(+)CXCR3(+) T lymphocytes in human colorectal carcinoma. Int J Cancer 2005;116:949-956.

131. Vaday GG, Peehl DM, Kadam PA, Lawrence DM. Expression of CCL5 (RANTES) and CCR5 in prostate cancer. Prostate 2006;66:124-134.

132. van Deventer HW, O'Connor W, Jr., Brickey WJ, Aris RM, Ting JP, Serody JS. C-C chemokine receptor 5 on stromal cells promotes pulmonary metastasis. Cancer Res 2005;65:3374-3379.

133. Sugasawa H, Ichikura T, Tsujimoto H, *et al*. Prognostic significance of expression of CCL5/ RANTES receptors in patients with gastric cancer. J Surg Oncol 2008;97:445-450.

134. Manes S, Mira E, Colomer R, *et al*. CCR5 expression influences the progression of human breast cancer in a p53-dependent manner. J Exp Med 2003;198:1381-1389.

135. Aldinucci D, Lorenzon D, Cattaruzza L, *et al*. Expression of CCR5 receptors on Reed-Sternberg cells and Hodgkin lymphoma cell lines: involvement of CCL5/ Rantes in tumour cell growth and microenvironmental interactions. Int J Cancer 2008;122:769-776.

136. Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice? Transfus Med 2001;11:403-417.

137. Kim JJ, Nottingham LK, Sin JI, *et al*. CD8 positive T cells influence antigen-specific immune responses through the expression of chemokines. J Clin Invest 1998;102: 1112-1124.

138. Taub DD, Ortaldo JR, Turcovski-Corrales SM, Key ML, Longo DL, Murphy WJ. Beta chemokines costimulate lymphocyte cytolysis, proliferation, and lymphokine production. J Leukoc Biol 1996;59:81-89.

139. Azenshtein E, Luboshits G, Shina S, *et al*. The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. Cancer Res 2002;62:1093-1102.

140. Robinson SC, Scott KA, Balkwill FR. Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNFalpha. Eur J Immunol 2002;32:404-412.

141. Wu Y, Li YY, Matsushima K, Baba T, Mukaida N. CCL3-CCR5 axis regulates intratumoural accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis process. J Immunol 2008;181:6384-6393.

142. van Deventer HW, Wu QP, Bergstralh DT, *et al*. C-C chemokine receptor 5 on pulmonary fibrocytes facilitates migration and promotes metastasis via matrix metalloproteinase 9. Am J Pathol 2008;173:253-264.

143. Tan MC, Goedegebuure PS, Belt BA, *et al*. Disruption of CCR5-dependent homing of regulatory T cells inhibits tumour growth in a murine model of pancreatic cancer. J Immunol 2009;182:1746-1755.

144. Samson M, Libert F, Doranz BJ, *et al*. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722-725.

145. Dorr P, Westby M, Dobbs S, *et al*. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob Agents Chemother 2005;49:4721-4732.

146. Maeda K, Yoshimura K, Shibayama S, *et al*. Novel low molecular weight spirodiketopiperazine derivatives potently inhibit R5 HIV-1 infection through their antagonistic effects on CCR5. J Biol Chem 2001;276:35194-35200.

147. Strizki JM, Xu S, Wagner NE, *et al*. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection *in vitro* and *in vivo*. Proc Natl Acad Sci USA 2001;98:12718-12723.

148. Baba M, Nishimura O, Kanzaki N, *et al*. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci USA 1999;96:5698-5703.

149. Wilkin TJ, Su Z, Kuritzkes DR, *et al*. HIV type 1 chemokine coreceptor use among antiretroviralexperienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. Clin Infect Dis 2007;44:591-595.

150. Hutter G, Nowak D, Mossner M, *et al*. Long-term control of HIV by CCR5 Delta32/Delta32 stemcell transplantation. N Engl J Med 2009;360:692-698.

151. Takanami I. Overexpression of CCR7 mRNA in nonsmall cell lung cancer: correlation with lymph node metastasis. Int J Cancer 2003;105:186-189.

152. Mashino K, Sadanaga N, Yamaguchi H, *et al*. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Res 2002;62: 2937-2941.

153. Kodama J, Hasengaowa, Kusumoto T, *et al*. Association of CXCR4 and CCR7 chemokine receptor expression and lymph node metastasis in human cervical cancer. Ann Oncol 2007;18:70-76.

154. Hedrick JA, Zlotnik A. Identification and characterization of a novel beta chemokine containing six conserved cysteines. J Immunol 1997;159:1589-1593.

155. Gunn MD, Kyuwa S, Tam C, *et al*. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J Exp Med 1999;189:451-460.

156. Cabioglu N, Yazici MS, Arun B, *et al*. CCR7 and CXCR4 as novel biomarkers predicting axillary lymph node metastasis in T1 breast cancer. Clin Cancer Res 2005;11:5686-5693.

157. Takeuchi H, Fujimoto A, Tanaka M, Yamano T, Hsueh E, Hoon DS. CCL21 chemokine regulates chemokine receptor CCR7 bearing malignant melanoma cells. Clin Cancer Res 2004;10:2351-2358.

158. Wiley HE, Gonzalez EB, Maki W, Wu MT, Hwang ST. Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. J Natl Cancer Inst 2001;93:1638-1643.

159. Gunther K, Leier J, Henning G, *et al*. Prediction of lymph node metastasis in colorectal carcinoma by expression of chemokine receptor CCR7. Int J Cancer 2005;116:726-733.

160. Wang J, Xi L, Gooding W, Godfrey TE, Ferris RL. Chemokine receptors 6 and 7 identify a metastatic expression pattern in squamous cell carcinoma of the head and neck. Adv Otorhinolaryngol 2005;62:121-133.

161. Heresi GA, Wang J, Taichman R, *et al*. Expression of the chemokine receptor CCR7 in prostate cancer presenting with generalized lymphadenopathy: report of a case, review of the literature, and analysis of chemokine receptor expression. Urol Oncol 2005;23:261-267.

162. Ding Y, Shimada Y, Maeda M, *et al*. Association of CC chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. Clin Cancer Res 2003;9:3406-3412.

163. Yan C, Zhu ZG, Yu YY, *et al*. Expression of vascular endothelial growth factor C and chemokine receptor CCR7 in gastric carcinoma and their values in predicting lymph node metastasis. World J Gastroenterol 2004;10:783-790.

164. Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA. Autologous chemotaxis as a mechanism of tumour cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. Cancer Cell 2007;11:526-538.

165. Fonso-Perez M, Lopez-Giral S, Quintana NE, Loscertales J, Martin-Jimenez P, Munoz C. Anti-CCR7 monoclonal antibodies as a novel tool for the treatment of chronic lymphocyte leukaemia. J Leukoc Biol 2006;79:1157-1165.

166. Bajetto A, Barbieri F, Dorcaratto A, *et al*. Expression of CXC chemokine receptors 1-5 and their ligands in human glioma tissues: role of CXCR4 and SDF1 in glioma cell proliferation and migration. Neurochem Int 2006;49:423-432.

167. Murphy C, McGurk M, Pettigrew J, *et al*. Nonapical and cytoplasmic expression of interleukin-8, CXCR1, and CXCR2 correlates with cell proliferation and microvessel density in prostate cancer. Clin Cancer Res 2005;11:4117-4127.

168. Mestas J, Burdick MD, Reckamp K, Pantuck A, Figlin RA, Strieter RM. The role of CXCR2/ CXCR2 ligand biological axis in renal cell carcinoma. J Immunol 2005;175:5351-5357.

169. Richards BL, Eisma RJ, Spiro JD, Lindquist RL, Kreutzer DL. Coexpression of interleukin-8 receptors in head and neck squamous cell carcinoma. Am J Surg 1997;174:507-512.

170. Smith DR, Polverini PJ, Kunkel SL, *et al*. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J Exp Med 1994;179:1409- 1415.

171. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumourigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest 1996;97:2792-2802.

172. Arenberg DA, Keane MP, DiGiovine B, *et al*. Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 1998;102:465-472.

173. Zhu YM, Bagstaff SM, Woll PJ. Production and upregulation of granulocyte chemotactic protein-2/CXCL6 by IL-1β and hypoxia in small cell lung cancer. Br J Cancer 2006;94:1936- 1941.

174. Yuan A, Yang PC, Yu CJ, *et al*. Interleukin-8 messenger ribonucleic acid expression correlates with tumour progression, tumour angiogenesis, patient survival, and timing of relapse in non-small-cell lung cancer. Am J Respir Crit Care Med 2000;162:1957-1963.

175. Masuya D, Huang C, Liu D, *et al*. The intratumoural expression of vascular endothelial growth factor and interleukin-8 associated with angiogenesis in nonsmall cell lung carcinoma patients. Cancer 2001;92:2628- 2638.

176. Orditura M, De VF, Catalano G, *et al*. Elevated serum levels of interleukin-8 in advanced non-small cell lung cancer patients: relationship with prognosis. J Interferon Cytokine Res 2002;22:1129-1135.

177. White ES, Flaherty KR, Carskadon S, *et al*. Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. Clin Cancer Res 2003;9:853-860.

178. Chen JJ, Yao PL, Yuan A, *et al*. Up-regulation of tumour interleukin-8 expression by infiltrating macrophages: its correlation with tumour angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res 2003;9:729-737.

179. Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst 1998;90:447-454.

180. Fregene TA, Khanuja PS, Noto AC, *et al*. Tumour-associated angiogenesis in prostate cancer. Anticancer Res 1993;13:2377-2381.

181. Inoue K, Slaton JW, Eve BY, *et al*. Interleukin 8 expression regulates tumourigenicity and metastases in androgen-independent prostate cancer. Clin Cancer Res 2000;6:2104-2119.

182. Inoue K, Slaton JW, Kim SJ, *et al*. Interleukin 8 expression regulates tumourigenicity and metastasis in human bladder cancer. Cancer Res 2000;60:2290-2299.

183. Merogi AJ, Marrogi AJ, Ramesh R, Robinson WR, Fermin CD, Freeman SM. Tumour-host interaction: analysis of cytokines, growth factors, and tumour-infiltrating lymphocytes in ovarian carcinomas. Hum Pathol 1997;28:321-331.

184. Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ. Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res 1994;54:3242-3247.

185. Ugurel S, Rappl G, Tilgen W, Reinhold U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumour progression and survival. J Clin Oncol 2001;19:577-583.

186. Varney ML, Johansson SL, Singh RK. Distinct expression of CXCL8 and its receptors CXCR1 and CXCR2 and their association with vessel density and aggressiveness in malignant melanoma. Am J Clin Pathol 2006;125: 209-216.

187. Huang S, Mills L, Mian B, *et al*. Fully humanized neutralizing antibodies to interleukin-8 (ABXIL8) inhibit angiogenesis, tumour growth, and metastasis of human melanoma. Am J Pathol 2002;161:125-134.

188. Shi Q, Abbruzzese JL, Huang S, Fidler IJ, Xiong Q, Xie K. Constitutive and inducible interleukin 8 expression by hypoxia and acidosis renders human pancreatic cancer cells more tumourigenic and metastatic. Clin Cancer Res 1999;5:3711-3721.

189. Karashima T, Sweeney P, Kamat A, *et al*. Nuclear factor-kappaB mediates angiogenesis and metastasis of human bladder cancer through the regulation of interleukin-8. Clin Cancer Res 2003;9:2786-2797.

190. Mian BM, Dinney CP, Bermejo CE, *et al*. Fully human anti-interleukin 8 antibody inhibits tumour growth in orthotopic bladder cancer xenografts via downregulation of matrix metalloproteases and nuclear factor-kappaB. Clin Cancer Res 2003;9:3167-3175.

191. Bendre MS, Margulies AG, Walser B, *et al*. Tumour-derived interleukin-8 stimulates osteolysis independent of the receptor activator of nuclear factorkappaB ligand pathway. Cancer Res 2005;65:11001-11009.

192. Charafe-Jauffret E, Ginestier C, Iovino F, *et al*. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res 2009;69:1302-1313.

193. Collins TS, Lee LF, Ting JP. Paclitaxel up-regulates interleukin-8 synthesis in human lung carcinoma through an NF-kappaB- and AP-1-dependent mechanism. Cancer Immunol Immunother 2000;49:78-84.

194. De Larco JE, Wuertz BR, Manivel JC, Furcht LT. Progression and enhancement of metastatic potential after exposure of tumour cells to chemotherapeutic agents. Cancer Res 2001;61:2857-2861.

195. Lev DC, Onn A, Melinkova VO, *et al*. Exposure of melanoma cells to dacarbazine results in enhanced tumour growth and metastasis *in vivo*. J Clin Oncol 2004;22:2092- 2100.

196. Wilson C, Purcell C, Seaton A, *et al*. Chemotherapy-induced CXC-chemokine/ CXC-chemokine receptor signaling in metastatic prostate cancer cells confers resistance to oxaliplatin through potentiation of nuclear factorkappaB transcription and evasion of apoptosis. J Pharmacol Exp Ther 2008;327:746-759.

197. Merritt WM, Lin YG, Spannuth WA, *et al*. Effect of interleukin-8 gene silencing with liposome-encapsulated small interfering RNA on ovarian cancer cell growth. J Natl Cancer Inst 2008;100:359-372.

198. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clin Cancer Res 2008;14:6735-6741.

199. Winters MP, Crysler C, Subasinghe N, *et al*. Carboxylic acid bioisosteres acylsulfonamides, acylsulfamides, and sulfonylureas as novel antagonists of the CXCR2 receptor. Bioorg Med Chem Lett 2008;18:1926-1930.

200. Lai G, Merritt JR, He Z, *et al*. Synthesis and structure-activity relationships of new disubstituted phenyl-containing 3,4-diamino-3-cyclobutene-1,2-diones as CXCR2 receptor antagonists. Bioorg Med Chem Lett 2008;18:1864-1868.

201. Walters I, Austin C, Austin R, *et al*. Evaluation of a series of bicyclic CXCR2 antagonists. Bioorg Med Chem Lett 2008;18:798-803.

202. Gonsiorek W, Fan X, Hesk D, *et al*. Pharmacological characterization of Sch527123, a potent allosteric CXCR1/ CXCR2 antagonist. J Pharmacol Exp Ther 2007;322:477-485.

203. McCleland BW, Davis RS, Palovich MR, *et al*. Comparison of N,N'-diarylsquaramides and N,N'-diarylureas as antagonists of the CXCR2 chemokine receptor. Bioorg Med Chem Lett 2007;17:1713-1717.

204. Dwyer MP, Yu Y, Chao J, *et al*. Discovery of 2-hydroxy-N,N-dimethyl-3-{2-[[(R)-1-(5-methylfuran-2- yl)propyl]amino]-3,4-dioxocyclobut-1-enylamino}benzamide (SCH 527123): a potent, orally bioavailable CXCR2/ CXCR1 receptor antagonist. J Med Chem 2006;49:7603-7606.

205. Zhu YM, Webster SJ, Flower D, Woll PJ. Interleukin-8/CXCL8 is a growth factor for human lung cancer cells. Br J Cancer 2004;91:1970-1976.

206. Moore BB, Arenberg DA, Stoy K, *et al*. Distinct CXC chemokines mediate tumourigenicity of prostate cancer cells. Am J Pathol 1999;154:1503-1512.

207. Kleinhans M, Tun-Kyi A, Gilliet M, *et al*. Functional expression of the eotaxin receptor CCR3 in CD30+ cutaneous T-cell lymphoma. Blood 2003;101:1487-1493.

208. Tanaka T, Bai Z, Srinoulprasert Y, Yang BG, Hayasaka H, Miyasaka M. Chemokines in tumour progression and metastasis. Cancer Sci 2005;96:317-322.

209. Burkle A, Niedermeier M, Schmitt-Graff A, Wierda WG, Keating MJ, Burger JA. Overexpression of the CXCR5 chemokine receptor, and its ligand, CXCL13 in B-cell chronic lymphocytic leukaemia. Blood 2007;110:3316-3325.

210. Andreasson U, Ek S, Merz H, *et al*. B cell lymphomas express CX3CR1 a non-B cell lineage adhesion molecule. Cancer Lett 2008;259:138-145.

211. Panse J, Friedrichs K, Marx A, *et al*. Chemokine CXCL13 is overexpressed in the tumour tissue and in the peripheral blood of breast cancer patients. Br J Cancer 2008;99:930-938.

212. Meijer J, Ogink J, Kreike B, Nuyten D, de Visser KE, Roos E. The chemokine receptor CXCR6 and its ligand CXCL16 are expressed in carcinomas and inhibit proliferation. Cancer Res 2008;68:4701-4708.

213. Milliken D, Scotton C, Raju S, Balkwill F, Wilson J. Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. Clin Cancer Res 2002;8: 1108-1114.

214. Li X, Loberg R, Liao J, *et al*. A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. Cancer Res 2009;69:1685-1692.

215. Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. Cancer Res 2004;64:4693-4698.

216. Ghadjar P, Loddenkemper C, Coupland SE, *et al*. Chemokine receptor CCR6 expression level and aggressiveness of prostate cancer. J Cancer Res Clin Oncol 2008;134:1181-1189.

217. Gutwein P, Schramme A, Sinke N, *et al*. Tumoural CXCL16 expression is a novel prognostic marker of longer survival times in renal cell cancer patients. Eur J Cancer 2009;45:478-489.

218. Ohta M, Kitadai Y, Tanaka S, *et al*. Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumour vascularity in human esophageal squamous cell carcinomas. Int J Cancer 2002;102:220-224.

219. Monti P, Leone BE, Marchesi F, *et al*. The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of antimalignant activity. Cancer Res 2003;63:7451-7461.

220. Cheadle EJ, Riyad K, Subar D, *et al*. Eotaxin-2 and colorectal cancer: a potential target for immune therapy. Clin Cancer Res 2007;13:5719-5728.

221. Ghadjar P, Coupland SE, Na IK, *et al*. Chemokine receptor CCR6 expression level and liver metastases in colorectal cancer. J Clin Oncol 2006;24:1910-1916.

222. Huang J, Hu J, Bian X, *et al*. Transactivation of the epidermal growth factor receptor by formylpeptide receptor exacerbates the malignant behaviour of human glioblastoma cells. Cancer Res 2007;67:5906-5913.

223. Huang J, Chen K, Gong W, *et al*. Receptor “hijacking” by malignant glioma cells: a tactic for tumour progression. Cancer Lett 2008;267:254-261.

224. Rabiet MJ, Huet E, Boulay F. Human mitochondria-derived Nformylated peptides are novel agonists equally active on FPR and FPRL1, while Listeria monocytogenes-derived peptides preferentially activate FPR. Eur J Immunol 2005;35:2486-2495.

225. Xia SH, Hu LP, Hu H, *et al*. Three isoforms of annexin I are preferentially expressed in normal esophageal epithelia but down-regulated in esophageal squamous cell carcinomas. Oncogene 2002;21:6641-6648.

226. Sun R, Iribarren P, Zhang N, *et al*. Identification of neutrophil granule protein cathepsin G as a novel chemotactic agonist for the G protein-coupled formyl peptide receptor. J Immunol 2004;173:428-436.

227. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. Trends Immunol 2002;23:541-548.

228. Rescher U, Danielczyk A, Markoff A, Gerke V. Functional activation of the formyl peptide receptor by a new endogenous ligand in human lung A549 cells. J Immunol 2002;169:1500-1504.

229. McCoy R, Haviland DL, Molmenti EP, Ziambaras T, Wetsel RA, Perlmutter DH. N-formylpeptide and complement C5a receptors are expressed in liver cells and mediate hepatic acute phase gene regulation. J Exp Med 1995;182:207-217.

230. Zhou Y, Bian X, Le Y, *et al*. Formylpeptide receptor FPR and the rapid growth of malignant human gliomas. J Natl Cancer Inst 2005;97:823-835.

Abbreviations

AIDS = Acquired immunodeficiency syndrome

AP = Aortapulmonary

bFGF = Basic fibroblast growth factor

BM = Bone marrow

CLL = Chronic lymphocytic leukaemia

CML = Chronic myelogenous leukaemia

CNS = Central nervous system

CR = Cajal Retzius

CRC = Colorectal carcinoma

CSC = Cancer stem cell

DFS = Disease free survival

DG = Dentate gyrus

ECM = Extra cellular matrix

fMLP = N-formyl-methionyl-leucyl-phenylalanine

FPR = Formylated peptide receptor

GAG = Glycosaminoglycan

GBM = Glioblastoma multiforme

GPCR = G-protein coupled receptor

HAART = Highly active anti-retroviral therapy

HIV = Human immunodeficiency virus

HPC = Hematopoietic progenitor cell

H-RS = Hodgkin and Reed Sternberg

HSC = Hematopoietic stem cell

ICSBP = Interferon consensus sequence binding protein

IFN = Interferon

IL-2 = Interleukin 2

LTB4 = Leukotriene B4

MALT = Mucosa associated lymphoid tissue

MAPK = Mitogen-activated protein kinases

MMP = Matrix metalloproteinase

MPC = Myeloid progenitor cell

NB = Neuroblastoma

NK-cell = Natural killer cell

OPC = Oligodendrocyte precursor cell

OS = Overall survival

PAK = Platelet activating factor

PBMC = Peripheral blood mononuclear cell

PCA = Prostate cancer

PDAC = Pancreatic ductal adenocarcinoma

PI3K = Phosphatidylinositol 3-kinase

PKC = Protein kinase C

PLC = Phospholipase C

RCC = Renal cell carcinoma

SCID = Severe combined immunodeficiency

shRNA = Small hairpin RNA

siRNA = Short interference RNA

SLL = Small lymphocytic lymphoma

SMZL = Splenic marginal zone lymphoma

(S)VZ = (sub) ventricular zone

TNF = Tumour necrosis factor

VEGF = Vascular endothelial growth factor

Table 1 | The systemic and original chemokine nomenclature

Systemic name	Original name
CXCL1	GROα growth related oncogene α
CXCL2	GROβ growth related oncogene β
CXCL3	GROγ growth related oncogene γ
CXCL4	PF-4 platelet factor 4
CXCL5	ENA-78 epithelial cell derived neutrophil activating factor 78
CXCL6	GCP-2 granulocyte chemoattractant protein 2
CXCL7	NAP-2 neutrophil activating protein 2
CXCL8	IL-8 interleukin 8
CXCL9	MIG monokine induced by interferon-γ
CXCL10	IP-10 γ interferon inducible protein 10
CXCL11	I-TAC interferon inducible T cell α-chemoattractant
CXCL12	SDF-1α/β stromal cell derived factor 1
CXCL13	BCA-1-B cell avtivating chemokine 1
CXCL14	BRAK breast and kidney chemokine
CXCL15	Lungkine
CXCL16	SR-PSOX scavenger receptor that binds phosphatidyserine and oxidized lipoprotein
CCL1	I-309
CCL2	MCP-1 monocyte chemoattractant protein 1
CCL3	MIP-1α macrophage inflammatory protein 1α
CCL3L1	LD-78β
CCL4	MIP-1β macrophage inflammatory protein 1β
CCL5	RANTES regulated on activation, normally T cell expressed and secreted
CCL6	C10
CCL7	MCP-3 monocyte chemoattractant protein 3
CCL8	MCP-2 monocyte chemoattractant protein 2
CCL9/10	MIP-1γ macrophage inflammatory protein 1γ
CCL11	Eotaxin
CCL12	MCP-5 monocyte chemoattractant protein 5
CCL13	MCP-4 monocyte chemoattractant protein 4
CCL14	HCC-1 hemofiltrate CC chemokine
CCL15	Lkn-1 leukotactin 1 / HCC-2 hemofiltrate CC chemokine
CCL16	LEC liver expressed chemokine / HCC-4 hemofiltrate CC chemokine
CCL17	TARC thymus and activation regulated chemokine
CCL18	PARC pulmonary and activation regulated chemokine
CCL19	ELC Epstein-Barr virus induced receptor ligand chemokine
CCL20	LARC liver and activation regulated chemokine / MIP-3α macrophage inflammatory protein 3α

Table 1 continued

Systemic name	Original name
CCL21	SLC secondary lymphoid tissue chemokine
CCL22	MDC macrophage derived chemokine
CCL23	MPIF-1 myeloid progenitor inhibitory factor 1
CCL24	MPIF-2 myeloid progenitor inhibitory factor 2 / Eotaxin-2
CCL25	TECK thymus expressed chemokine
CCL26	Eotaxin-3
CCL27	Eskine / CTACK cutaneous T cell-attracting chemokine
CCL28	MEC mucosa-associated epithelial chemokine
XCL1	Lymphotactin-α
XCL2	Lymphotactin-β / SCM-1β single C motif-1β
CX3CL1	Fractalkine

Table 2 | An overview of the chemokine subfamilies

Family	Structure	Receptor	Ligand
CXC (α)	NH2--C-X-C– 2 disulphide bridges	CXCR1	CXCL6-8
		CXCR2	CXCL1-3, CXCL5-8
		CXCR3	CXCL9-11
		CXCR4	CXCL12
		CXCR5	CXCL13
		CXCR6	CXCL16
		CXCR7	CXCL12
CC (β)	NH2--C-C– 2 disulphide bridges	CCR1	CCL3, CCL3L1, CCL5, CCL9-10, CCL14-16
		CCR2	CCL2, CCL7-8, CCL12-13
		CCR3	CCL5-6, CCL11, CCL15-16, CCL23-24, CCL26
		CCR4	CCL17, CCL22
		CCR5	CCL3-4, CCL3L1, CCL5-6, CCL8, CCL12
		CCR6	CCL20
		CCR7	CCL19, CCL21
		CCR8	CCL1
		CCR9	CCL25
		CCR10	CCL27-28
C / XC (γ)	NH2--C– 1 disulphide bridge	XCR1	XCL1-2
CX3C (δ)	NH2--C-X-X-X– 2 disulphide bridges Mucin like stalk	CX3CR1	CX3CL1

Table 3 | Chemokine (receptor) agonists and antagonists

Drug name	Mechanism of Action	Indication	Study phase
SB656933	CXCR2	COPD, cystic fibrosis, ulcerative colitis	1, 2
SCH527123	CXCR2	asthma, COPD, psoriasis	2
Reparixin	CXCL8	ischemia-reperfusion injury after kidney/lung transplantation	2
MDX-1100	CXCL10	ulcerative colitis	1, 2
TG-0054	CXCR4	stem cell mobilization	1
AMD3100	CXCR4	stem cell mobilization in haematologic malignancies	1, 2, 3
AMD3100	CXCR4	Fanconi anemia	1, 2
AMD3100	CXCR4	stem cell mobilization	1, 2
AMD3100	CXCR4	lymphoma, Non-Hodgkin lymphoma (stem cell mobilization)	1, 2
AMD3100	CXCR4	relapsed or refractory acute myeloid leukaemia	1, 2
AMD(11)070	CXCR4	human immunodeficiency virus	1, 2
SP01A	CXCR4, CCR5	human immunodeficiency virus	1, 2
SH T 04268H (ZK811752)	CCR1	endometriosis	2
MLN1202	CCR2	atherosclerosis	2
BMS-741672	CCR2	Diabetes Mellitus, neuropathic pain	2
OPL-CCL2-LPM	CCL2	IgA nephropathy, proteinuria	1
TPI ASM8	CCR3	asthma	2
KW-0761	CCR4	Peripheral/cutaneous T-Cell Lymphoma/ leukaemia	1, 2
Vicriviroc (Sch-D)	CCR5	human immunodeficiency virus	1, 2, 3
Maraviroc	CCR5	human immunodeficiency virus	2, 3, 4
PF-00232798	CCR5	human immunodeficiency virus	2
GSK706769	CCR5	human immunodeficiency virus	1
PRO140	CCR5	human immunodeficiency virus	(1), 2
GW873140	CCR5	human immunodeficiency virus	2
INCB009471	CCR5	human immunodeficiency virus	2
ZFN modified T-cells	CCR5	human immunodeficiency virus	1
CCR5mAb004	CCR5	human immunodeficiency virus	1
DC pulsed with pp65 RNA	CCR7	glioblastoma, citomegalovirus	1
DC/apo-Nec	CCR7	melanoma	1
Autologous DC-adenovirus	CCL21	metastatic melanoma	1
CCL21 vaccine	CCL21	lung cancer	1

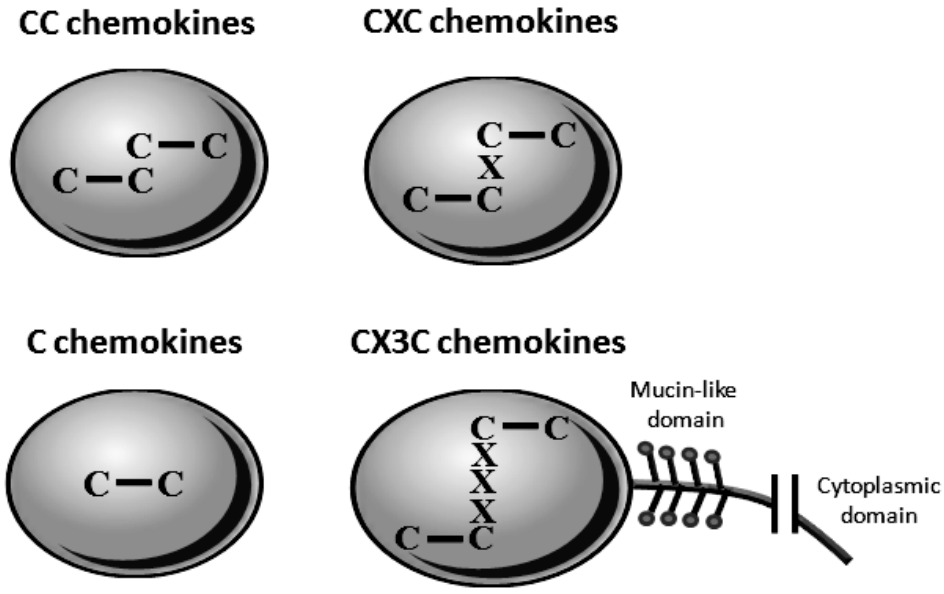

Figure 1 | Structure of chemokine classes

An overview of the four subfamilies of chemokines and their (chemical)structure

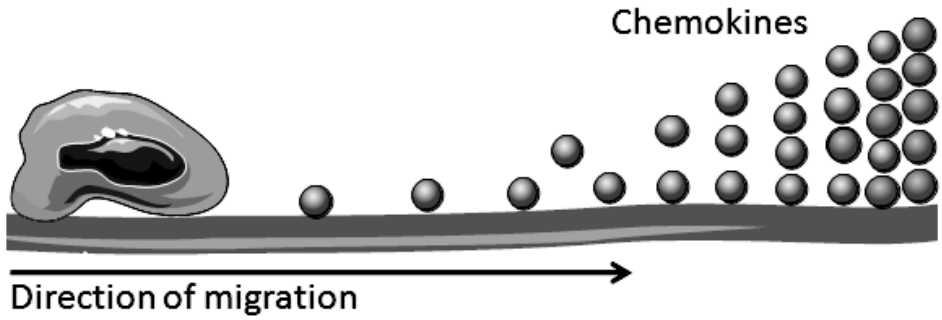

Figure 2 | Chemokine induced chemotaxis

Cells expressing a chemokine receptor migrate along a chemokine gradient.

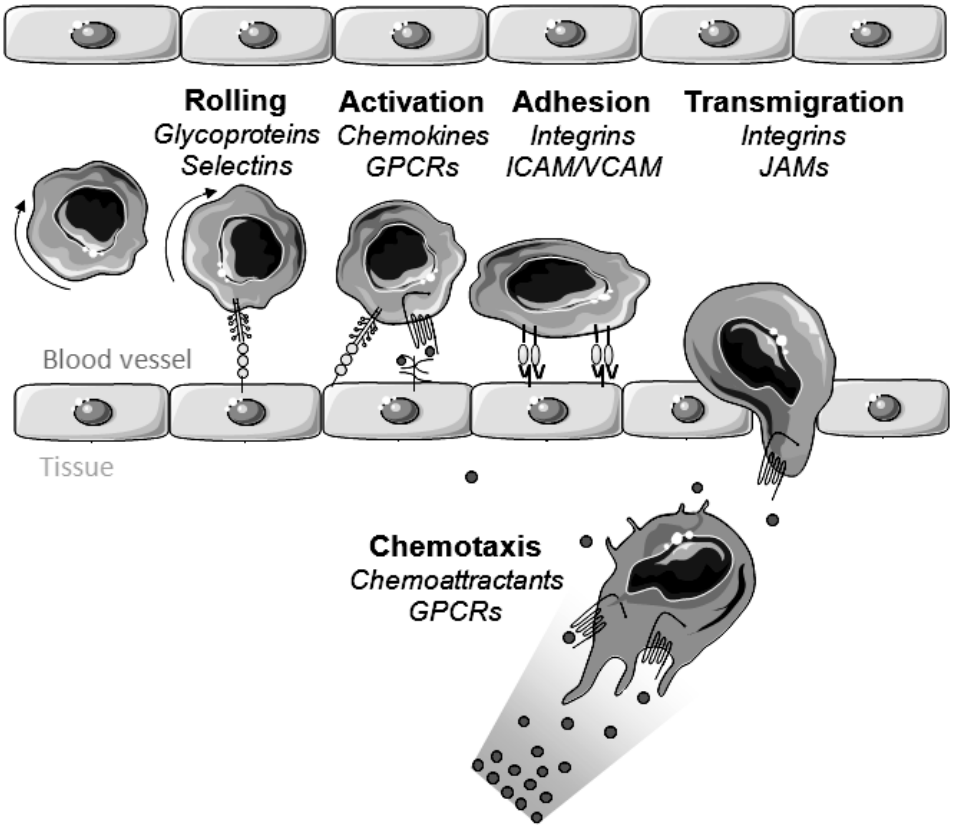

Figure 3 |Cell migration and extravasation

A schematic overview of molecules involved in cell migration and extravasation. Figures were produced using Servier Medical Art.

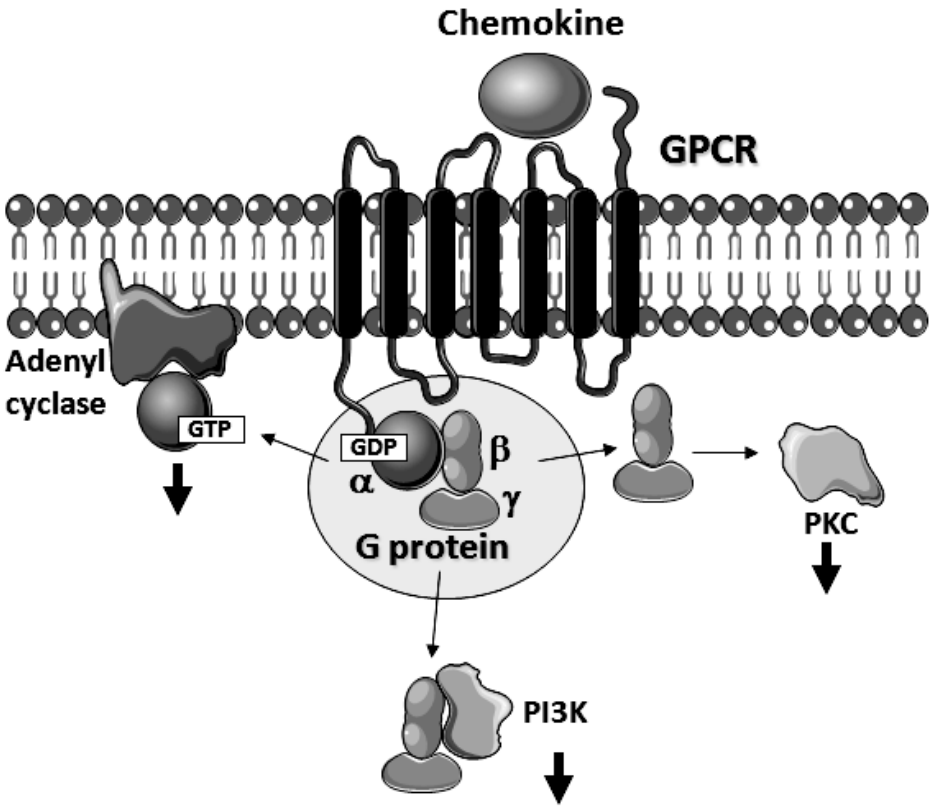

Figure 4 | GPCR signaling

Downstream signaling pathway of GPCRs
Figures were produced using Servier Medical